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WITH SIXTY-TWO PLATES AND ONE HUNDRED AND FORTY-TWO TEXT FIGURES

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## ERRATA, VOLUME XII

Page 193, line 6. For *Fremanii*, read *Freemanii*.

Page 345, 10th line from bottom. For *littorales*, read *littoralis*.

Page 501, top of page. *July* should be *October*.

Page 605, line 21. For *Untrs.*, read *Unters*.

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## A STUDY OF SYNZESIS AND SYNAPSIS IN *LILIUM SUPERBUM* L.<sup>1</sup>

RUTH HAYES CHIPMAN

(Received for publication January 9, 1924)

The investigations to be described in this paper were undertaken at the suggestion of Dr. Bradley M. Davis to compare the history of the reduction division in *Lilium superbum* L. with the results of the study of Charles E. Allen (1905) on *Lilium canadense* L. and with conclusions of other authors on *Lilium* and related forms. The writer has found her preparations to be very like those of Dr. Allen, who kindly sent for comparison slides which furnished material for the figures accompanying his paper. The interpretation of the complex conditions present at certain stages of the reduction history is a matter of fundamental interest in biology, and this subject has furnished the chief problems of this study. It is a pleasure to me to acknowledge my indebtedness to Dr. Davis for material used in this study, and for the helpful interest which he has shown throughout its progress.

### HISTORICAL ACCOUNT

The genus *Lilium* has furnished material for cytological research since it was first used by Strasburger (1880). A history of cytological research on *Lilium* then is of particular interest as matter around which to build a history of cytological advance. As other material more suitable for certain phases of mitosis has furnished new clues, investigators have repeatedly returned to the lily for reconsideration.

Guignard (1884) published a description of the reduction division of *Lilium Martagon* which he said differed from *Allium* chiefly in the number of nuclear elements (chromosomes). He gave also thirteen figures of *Lilium superbum*. In this report he outlined the process of nuclear reduction as follows: a spireme stage; segmentation of the filament; nuclear-plate figure; longitudinal division of the elements (chromosomes) of the nuclear plate; star, and finally the spireme stages of the daughter nuclei. He pointed out the likeness of the process to that described by Flemming for

<sup>1</sup> Papers from the Department of Botany of the University of Michigan, no. 201.

animal cells and commented on the contraction figure which he thought was due to reagents.

Miss Sargent (1897) observed the first contraction figure in living pollen mother cells of *Lilium*. On the question of the tetrad formation Miss Sargent (1896) stated that during reduction in the embryo sac of *Lilium Martagon* the spireme does not segment into twelve parts which divide transversely, but that the chromosomes are split longitudinally during each of the three nuclear divisions which precede the formation of the ovum. She considered each chromosome to be composed of two segments tightly twisted about each other.

Mottier (1897) reported a transverse division of the chromosomes of *Lilium* to occur in the second of the two maturation divisions in the pollen mother cell. In this paper he discussed and figured the multipolar origin of the achromatic spindle. Strasburger and Mottier (1897) opposed the idea of a double longitudinal splitting, and the V- and U-shaped chromosomes appearing in the heterotypic anaphase were explained as a result of a folding together of the chromosomes which took place during, or previous to, metakinesis.

In 1897 Miss Sargent published her second paper on *Lilium*. She found twelve chromosomes to be formed from the spireme and to lie loosely in the nuclear cavity and that the two successive longitudinal divisions gave rise to four rows of granules in the strands. Miss Sargent described the second massing of chromatic material as a "second synapsis."

Strasburger (1900) returned to his former idea of two longitudinal divisions, the first of which he believed to occur during early prophase, the second at metaphase or early anaphase.

Dixon (1900) reported for the heterotypic mitosis in the lily the following stages: dolichonema, synapsis, strepsinema which arose by the looping of the dolichonema upon itself, and segmentation which resulted in loops or segments of the haploid number consisting of two short threads twisted together with four free ends.

Mottier in 1903 published his second paper on *Lilium*, wherein he reasserted his belief in the account of the prophases as given in his earlier paper, and stated that the spireme splits longitudinally and segments into chromosomes each consisting in *Lilium* of two rather long daughter segments.

Of the various interpretations of the reduction division, two theories, parasynapsis and telosynapsis, are conspicuous in recent literature. The theory of parasynapsis may be said to have its origin in the admirable work of Hans von Winiwarter (1900), and has since been supported by Grégoire, A. and K. E. Schreiner, Allen, and others. The theory of telosynapsis was advanced by Farmer and Moore (1905) and is supported by Digby, Fraser, Mottier, the students of *Oenothera*, and others. Both interpretations allow of the side-by-side position of the associating chromosomes, univalent in value, during and following the stage of diakinesis. Parasynapsis places the

time of association during leptonema and consummates the process in the first contraction. Telosynapsis reports the association as delayed until pachynema and consummates the process in the second contraction.

So many excellent reviews of literature and clear statements of the points in dispute between supporters of the parasynaptic and telosynaptic interpretations of the phenomena preceding reduction have been published since 1900 that more than an enumeration of some of the more important reviews themselves seems unnecessary: Farmer and Moore (1905), Allen (1905), Grégoire (1910), Digby (1919), Sharp (1920a, 1921).

It is generally conceded that there is a duality evident in the early spireme stage of the reduction division. That this duality is the result of a telophasic split due to centrally placed vacuoles in the telophasic chromosomes in the last somatic mitosis, is held by Fraser and Snell (1911), Fraser (1914), Digby (1919), and Nothnagel (1916). That the duality is due to the association of paternal and maternal leptotène threads, each thread representing a series of somatic chromosomes, is held by Grégoire (1905, 1907, 1910), Berghs (1904), Allen (1905), and many zoölogists.

Fraser and Snell (1911), Fraser (1914), Reed (1914), and Digby (1919) find alveolation of the chromosomes in the telophase preceding the first meiotic division to be so regular as to give rise to a true longitudinal split which persists through leptotène, into zygotène, and later reappears in the split pachytène stages.

Grégoire (1906) admits that frequently alveoli are distributed in a single row, but states that they often are abundant and may number several at the same level.

Sharp (1921, p. 149) states that

Careful study of the details of these telophasic changes shows that the alveolation proceeds with little regularity, and that each chromosome becomes an alveolate and then a reticulate body with nothing which can properly be called a longitudinal split.

Recently, Overton (1922) supports this conclusion of Sharp from studies on *Podophyllum*. There is some dispute as to the exact time at which alveolation begins. Miss Merriman (1904), Lundegårdh (1910), Némec (1910), and Reed (1914) report it as occurring as early as the anaphases in *Allium*, while Grégoire and Wygaerts (1903) found alveoli in *Trillium* even at metaphase.

Gates (1908, 1911), Geerts (1909, 1911), Davis (1910, 1911), and Cleland (1922) in *Oenothera* find only a single filament which segments during second contraction to give the diploid number of chromosomes. Recently, Gates and Rees (1921) have reported a single filament also for *Lactuca* which persists until segmentation at the second contraction. This is strong evidence for the theory of telosynapsis.

The cytological study of *Oenothera franciscana* made by Cleland (1922) is especially significant with reference to telosynapsis and parasynapsis. This species is also of special interest because its genetical history is known



as well as its cytology. *Oenothera franciscana*, described by H. H. Bartlett in 1914, has been grown in the gardens of B. M. Davis for eight generations. In this period of cultivation no departures from the type have been observed. One culture of 1373 plants was large enough to bring out variants if present in proportions such as are thrown by *O. Lamarckiana*. Davis (1922) presents *O. franciscana* as the purest *Oenothera* material known, and suggests that it be used as standard material with which forms may be mated in tests of cross breeding. Cleland (1922) finds evidence which leads him to a telosynaptic interpretation of the reduction prophase as found in the pollen mother cell. This material shows no general parallelism or significant side-by-side association of threads in early prophase. The pachynema is univalent and segments crosswise into the diploid number of chromosomes, which become associated in pairs largely through the formation of definite loops during second contraction. There is a perfect pairing of the chromosomes to form rings, which are arranged in a definite manner in striking contrast to the loose association of chromosomes characteristic of most *Oenothera* material. Thus, this cytological study strongly supports the genetical evidence for the high degree of purity of this species of *Oenothera*.

The stage of second contraction in its essentials, as it is now understood by the followers of the telosynaptic interpretation, was described and interpreted by Farmer and Moore (1905, p. 511). The detailed history of this critical stage, *i.e.*, second contraction, has been reworked and restated with little variation in essentials by Digby (1919), Fraser (1914), Mottier and Nothnagel (1913), and Nothnagel (1916). They hold that during the interval between synizesis and second contraction the original lengthwise fission in the spireme closes and may be seen only in exceptionally favorable cases. The spireme becomes rearranged and parts are pulled into parallel positions, the parallel portions being associating chromosome lengths. The relic of the original fission may be recognizable in one, or even in both, sides of the narrow V-shaped figures produced. Not all the bivalent chromosomes are produced in this way. Sometimes straight rodlets become associated. This massing, due to the rearrangement, then constitutes the second contraction stage.

Nothnagel (1916) emphasizes the bouquet stage, due to an entanglement of the spireme at the center of the nucleus, and the following phase of radiating loops extending to the periphery of the nucleus, which phase she calls "typical second contraction." One arm of a loop, she states, pairs with and twists about the arm of a neighboring loop. The crowding continues until only paired ends extending from the dense mass can be recognized.

Allen (1905), on the other hand, holding to parasynapsis, sees the persistence of the double-thread condition through to the second contraction and segmentation of the double thread resulting in the reduced number of

paired chromosome segments. The rearrangement of the twisted bivalent thread which is still unsegmented may give rise to twelve loops continuous with one another. The arms of the loops, however, are not associating univalent chromosomes. Each loop is bivalent in character throughout its length.

It will be noted that there are observers from both the telo- and parasynaptic schools who agree as to the occurrence of an apparent bouquet stage, the number of loops of which is haploid. The Farmer-Moore-Digby-Fraser group, holding to a telosynaptic interpretation, report a closing of the lengthwise split in the spireme before this stage. Grégoire and Allen, holding to parasynapsis, fail to observe such a merging of threads in their material. The telosynaptic group believe segmentation to occur at the center of the massed filaments and loops thus to be formed toward the periphery. Allen reports segmentation at the periphery. He sees no evidence that the arms of loops become chromosomes placed side by side by the closing of the loops.

The observational differences between the two schools allow of the following brief summary, the parasynaptic interpretation being compared with the telosynaptic. Either alveolation in the chromosomes of the last somatic mitosis is so irregular that nothing which can properly be called a fission results, or it is regular and gives rise to a lengthwise split. Before synzesis occurs, either a double-thread or a single-thread system is present. If a double-thread system is present, it has arisen either by approximation in pairs of the leptotène threads or as a result of condensation of the portions of a split univalent thread. Whether the interpretation is parasynaptic or telosynaptic, most observations report a fusion of threads during synzesis, the filament thus formed splitting almost immediately upon emergence.

In the period preceding the second contraction, according to parasynapsis, the lengthwise split persists, while, according to telosynapsis, it closes and the spireme enters the early second contraction stages as a single filament. The double pachynema of parasynapsis then segments directly into the haploid number of bivalent chromosomes, while the single filament of telosynapsis segments into univalent chromosome initials, in number corresponding to the diploid count. Segmentation of the double pachynema of parasynapsis gives free ends appearing in pairs at the periphery of the nucleus and very little evidence of loops, while by telosynapsis a bouquet stage would naturally result from the looping of the single filament previous to segmentation and conjugation. Under parasynapsis, the conjugation is observed as between associated lengths of the double pachynema, while by telosynapsis the conjugation is described as between segments of a single pachynema which by looping of pachynema or otherwise come to lie side by side.

In brief, the parasynaptic and telosynaptic differences in interpretation of the stages in question are as follows: The school of parasynapsis holds

that the double-thread system arises by the approximation of maternal and paternal threads, the school of telosynapsis that the double-thread system is due to a process of alveolation, instigated in the telophasic chromosomes, which proceeds with such regularity as to produce a double-thread system that persists in prophase. The first group interprets the double pachynema as bivalent, any segment of which would have its origin in two somatic chromosomes, the one maternal, the other paternal. The second group interprets pachynema as univalent, the double threads produced by alveolation becoming single through condensation and fusion and segmenting into single chromosomes. Parasynapsis completes an association of the paternal and maternal chromosomes at synizesis, telosynapsis at second contraction.

It was the author's problem to determine whether the stages in the prophases of the reduction division as seen in *Lilium superbum* more nearly resemble the series described by the parasynaptic school or that of the telosynaptic interpretation. Since the method of telophasic alveolation and the details of the history just preceding synizesis and second contraction contribute the chief factors responsible for the differences in interpretation, it was necessary to study intensively the telophase of the last somatic mitosis, the onset of synizesis, and the phases between emergence from synizesis through segmentation to second contraction. Later stages, while interesting as showing the results of the prophase activities, have no direct bearing upon the problem at hand.

#### MATERIAL AND METHODS

The studies were made with few exceptions from slides prepared by myself in the laboratories of the University of Michigan under the direction of Dr. Davis. The material for young stages was collected by W. R. Taylor at Westinghouse near Philadelphia, June 14 and June 24, 1920. The fixing fluids used for the earlier stages were Allen's modification of Bouin's fluid, strong Flemming, strong Flemming with 1 percent urea and 1 percent maltose added, strong Flemming diluted one half, and strong Flemming diluted one half with 1 percent urea and 1 percent maltose added. For the young stages, the last three fluids named gave the best results.

The material for later stages was collected by Dr. Davis July 5, 1919, and July 7, 1917, at Westinghouse. The fixing fluids employed were strong Flemming with 1 percent urea and 1 percent maltose added, strong Flemming with 0.5 percent urea and 0.5 percent lactose, strong Flemming with 1 percent maltose, and medium strength chrom-acetic acid; of these the first three were satisfactory. Of the stains used, iron-alum haematoxylin was most successful.

Sections were cut from two microns in thickness for alveolation and mid-synizesis stages to 33 microns for pachynema and the second contraction figures.

## OBSERVATIONS ON LILIUM SUPERBUM

A brief outline of the author's conclusions from these studies will be given previous to the descriptions of observations on the details of synzesis and synapsis in the pollen mother cells.

The chromosomes, in the daughter nuclei resulting from the last arche-spore division, enter the resting stage by a process of alveolation which is initiated through the appearance of a lengthwise series of alveoli in the chromosomes. In the slender chromosomes of *L. superbum* the first alveoli tend to lie in a single median row. Later, alveoli appear on either side of the axis and the chromosomes are resolved into irregular reticula occupying a peripheral position in the nucleus. Soon the limitations of individual reticula become indistinguishable because of the development of connecting threads. A period of nuclear growth follows. Throughout this growth period the chromatin is distributed peripherally as a very fine network. In the later resting stages the limits of the individual reticula are again distinguishable. It seems probable that the growth of the chromatin network does not keep pace with the increase in the surface of the nucleus and that the last-formed connecting threads, since they are most delicate, are first to be broken down. The reticula then resolve themselves into irregular single threads which, through condensation of the chromatin, tend to straighten. Further contraction throws portions of the threads into tight coils that resemble knots. These coiled and tangled threads are found in anthers, many nuclei of which are entering synzesis. The threads then pair. Well preserved nuclei in this phase are not easily found. A technique sufficiently good for other stages throws portions of these thin paired threads into masses joined by exceedingly delicate connections. Free ends of threads are not common, and the whole chromatic system appears to be a network because of numerous connections between the threads. No doubt the arrangement of the chromosomes at the last telophase determines in large measure their relative position in the chromatic net. The heads of a pair of chromosomes may adhere to any portion of another pair. The chromatic net thus enters synzesis. The paired condition of the threads in many cases can be demonstrated during synzesis, and in most cases is evident immediately upon emergence. This paired condition persists through second contraction. Just before second contraction the double thread segments into the haploid number of paired chromosomes. Some very clear evidence of a lengthwise split in each of the associated chromosomes has been found. Work on stages following second contraction has yielded only confirmatory evidence of studies already published.

## EVENTS LEADING TO SYNZESIS

The alveolation of the chromosomes of *Lilium superbum* commences in early telophase. No indications of alveoli were found in the anaphasic period, as reported by Reed, Merriman, Lundegårdh, and Němec for *Allium*

and by de Litardière for *Podophyllum*. The slender chromosomes of *Lilium superbum* commonly show an axial row of clearly outlined alveoli (Pl. I, fig. 1). Such a single series of alveoli regularly arranged was also reported by Grégoire and Wygaerts (1903), and Grégoire (1906). Somewhat later, other alveoli peripheral in position appear in the substance of the chromosomes (figs. 2, 3). De Litardière (1921) describes for *Podophyllum* an axial row of initial alveoli in each chromosome, separated from each other by thin partitions. At the poles, during telophasic changes, de Litardière reports the alveoli as increasing in number and dimensions and as becoming distributed irregularly in the chromosomes, much as I have observed them for *Lilium superbum*. Eventually the chromosomes become resolved into reticula. Figure 4 is presented as an illustration of exceptionally clearly defined reticula. At this advanced stage most nuclei show a general chromatic network because of numerous connecting threads which obscure the limiting spaces between individual reticula.

The material selected as most satisfactory for the study of the so-called resting stage was fixed for twenty-four hours in strong Flemming plus 1 percent urea. In young anthers (*i.e.*, anthers still showing a few metaphases of the last archesporial mitosis) the nuclei are comparatively small and the chromatic reticulum holds a peripheral position with the plasmosomes generally in contact. In older anthers (*i.e.*, those in which the nuclei have all entered the resting stage and are of about the same size as those entering synizesis) the plasmosomes are rounded and are distinctly not connected with the reticulum. In these older nuclei the chromatic reticulum displays a banded appearance (fig. 5). This figure was taken from an anther from 1920 material, treated with strong Flemming for eighteen hours. It seems evident that connections between reticula are breaking down in certain regions and that individual reticula are being delimited. Throughout the nucleus condensation has thrown the unit reticula into irregular threads. Some portions show the diamond-shaped openings emphasized by Fraser and Snell (1911) in *Vicia Faba*, and interpreted as the axial row of alveoli, persisting from the telophasic alveolation, which give rise to a true lengthwise split in pachynema. In *Lilium superbum*, however, condensation proceeds and resolves these reticula into single, not double, irregular threads (fig. 6), such as are described in somatic mitoses by Sharp (1920b, 1913) for *Tradescantia virginiana* and for *Vicia Faba*. Figure 6, with its chromatin in a more advanced state of condensation, logically follows the banded state represented in figure 5. Here the plasmosomes are distinctly separate from the chromatic reticulum. Condensation has proceeded to such a degree that the irregular threads are beginning to straighten. In some of the nuclei of the anther from which figure 6 was drawn, the chromatic reticulum had begun to draw away from the nuclear membrane thus giving the first indication of the inception of synizesis. Nuclei with the threads preserved as in figure 6 were found only in a very limited region at the ends of anthers

cut to allow rapid penetration of the fixing fluid. In the interior of the anther tight coils, appearing as in figures 6 and 8, are thrown by less perfect fixation into disorganized masses. It is my opinion that published figures of Allen and others representing the chromatin of this stage as flakes connected by extremely delicate threads, as in figure 10, Plate II, are taken from imperfectly fixed material, for it seems reasonable to assume that imperfect fixation may throw a thread system into flakes but not flakes into threads.

The author suggests the following as a possible explanation of the very early delimitation of the unit reticula at the beginning of condensation. During the telophasic alveolation the unit reticula take a peripheral position. Then follows the gradual enlargement of the pollen mother cell during the resting period preceding the heterotypic prophase. The growth in extent of the general reticulum may not keep pace with the increase of nuclear surface, and thus by the time the nucleus has reached its full size the chromatic network becomes again resolved into unit reticula by the breaking down of the delicate connecting threads. Condensation then throws these unit reticula into irregular threads which through further contraction straighten to give leptonema.

Figure 7, Plate I, presents a nucleus in the early leptotène stage. The parallelism of threads may indicate the beginning of pairing, but it seems more probable that it is chance association. Figure 8 is a stage of late leptonema. Here condensation has proceeded to such a degree that the threads have become tightly coiled and twisted in portions of their length. Such coils are shown only in material particularly well fixed and it seems probable that poor fixation would present such regions as irregular flakes. The threads are considerably thicker here than in the preceding stage. In the nucleus represented in figure 9 the fixation is not as satisfactory as that shown in figure 8. In several regions of the nucleus portions of the threads have been thrown into flakes, but the pairing of threads previous to synzesis seems to be quite evident. Figure 8 was drawn from an anther most of the nuclei of which were entering synzesis. This nucleus was selected from a small group of nuclei near the cut end of the anther. The nuclei in the interior and consequently less well fixed part of this anther had the appearance of figure 10, Plate II, *i.e.*, they showed flakes with here and there unmistakable pairs of leptotène threads.

The withdrawal of the chromatin from the periphery of the nucleus marks the beginning of synzesis. It is evident that the chromatin enters this stage as a reticulum, by which I mean that the paired leptotène threads adhere at various points. In sections 33 microns thick, free ends of threads could not be found. Free ends such as are shown in figure 11, Plate II, result from cutting. In figure 11 are two rather striking examples of paired leptotène threads attached at one point and rather widely separated elsewhere. Such cases lead one to believe that the homologous

chromosomes represented by sections of the paternal and maternal threads may pair first at one end and then approach each other throughout their length. However, the threads are extremely delicate, and their massing at this period makes observation difficult. That the nuclear cavity enlarges at this time is obvious, but there is an equally evident contraction of the chromatic network contrary to the views of Lawson (1911).

Favorable material shows threads on the surface of even the dense and much-contracted chromatin mass of mid-synizesis (fig. 12). Thin sections of nuclei in this stage reveal not only a thread system but a double-thread system (fig. 13). Figure 11, from material more satisfactorily fixed than that shown in figures 9 and 10, demonstrates quite clearly the independence of the paired threads even during the close association accompanying synizesis. Imperfect fixation may have been responsible for the figures often published showing the union of the two threads, as suggested in figure 20, Plate III. After some time the thread system begins to loosen, preliminary to emergence from synizesis (fig. 18). As shown in figures 14 and 18, there is clear evidence of paired threads. The author is convinced that in well fixed nuclei (fig. 19) the chromatin emerges as a double spireme and normally continues thus through pachynema. In the material used, very few nuclei could be found with the single thread commonly reported for the pachynema. A single spireme, then, such as is shown in figures 16, 16*a*, and 17, occurs seldom, or else is due to imperfect fixation. That the whole chromosome complex exists as a spireme which could be unraveled and made into a straight cord seems highly improbable. It is more reasonable to think of a netted structure with the ends of paired chromosomes adhering in some cases to ends of other pairs, but more often to the sides. This much is evident, that in sections 33 microns thick, which include an entire nucleus, no free ends of the spireme in the double pachynema stage could be found.

Of the various interpretations that might be offered for the presence of the single thread when found in the stages emerging from synizesis, two will be considered. First, it is possible that pairing, in some cases, is delayed and does not take place until after synizesis and therefore after condensation has proceeded to a marked degree. Evidence for this interpretation was found in the anther from which figures 16, 16*a*, and 17 have been taken. Figure 17 the author believes to be comparable to Miss Digby's (1919) figures 57, 58, and 59 for *Osmunda*, which she interprets as univalent threads pairing previous to the second contraction. Figure 15 shows at the right a case of pairing delayed until late synizesis or the early period of emergence. If pairing may be delayed until the stage of emergence from synizesis, why may it not be deferred in some cases until the pachytène stage? In such cases condensation would proceed to a marked degree before the threads completed pairing, and as a result the univalent threads themselves would have a thickness comparable to that usual for pachynema.

A second interpretation of the single pachynemal thread, suggested by its occasional extreme thickness, is that, because of imperfect fixation, the double threads have merged into a single structure in somewhat the same way that coils of threads during leptonema (fig. 8) may become represented by flakes (figs. 9, 10).

#### EVENTS LEADING TO SECOND CONTRACTION

Condensation continues regularly throughout the nucleus. As far as their position in the reticulum allows, the paired segments of the thread system lie at the surface. In *Lilium superbum* usually several pairs of these chromosome segments are drawn across the center of the nucleus, thus offering further evidence that the thread system is a reticulum.

Free ends of the thread system appear first in pairs at the periphery (fig. 21). The bouquet stage can be found in this material, but is not common. Its presence or absence seems to be connected with the time of the onset of the second contraction. If the massing, called "second contraction," has occurred previous to segmentation, then the looped arrangement naturally follows, but if segmentation takes place while the threads are at the periphery, then there should be no indication of loops. The chromosome segments of *Lilium superbum* are very long and lie across the interior of the nucleus or at the surface (fig. 22, Pl. IV). Usually seven or eight pairs of segments can be easily counted, but the remaining five or four pairs are very much entangled and can be followed only with difficulty. Good evidence for closed loops, which would support the telosynaptic interpretation at this point in the nuclear history, were not found. Loops, such as are shown in figures 26*a*, *b*, *c*, *d*, and *e*, were observed in sections 17 microns thick, but here the author believes the appearance to be due to a cutting of the twisted chromosome segments at an intersection as she was not able to find such loops in sections 33 microns thick.

If massing of the chromosomes has not commenced previous to segmentation, it follows almost immediately, and the chromosome segments enter the stage of second contraction (figs. 23, 24). The material studied shows some very clear evidence for the presence of a lengthwise split in each of the associating chromosomes at the time of second contraction (fig. 25), but these studies have not yet given convincing evidence of the exact time of the appearance of this fission which probably initiates the division of the chromosomes in the anaphase of the heterotypic mitosis.

The writer's conclusions may be expressed as follows: Alveolation of the chromosomes following the last somatic mitosis distributes the chromatin in the form of a reticulum at the surface of the nucleus. As the nuclei enlarge, the general reticulum becomes cut into unit reticula. The unit reticula resolve themselves into zigzag threads. Further condensation throws these threads at points into tight coils. The structure of the coils is easily destroyed by poor fixation, which condenses the chromatin at such



points into flakes. These threads show pairing previous to synizesis, but the author believes that pairing may sometimes be delayed until the chromatic filaments are emerging from synizesis. Most anthers, however, show, almost immediately upon emergence from synizesis, the separation of the two threads which have paired previous to or during synizesis. Well fixed material shows the paired threads independent of one another, and the author does not believe that they unite to form a single spireme. From this point on, the material shows no reliable evidence for the fusion of the associated threads. The apparently continuous double pachynema segments into the haploid number of paired chromosome initials. The first breaks appear at the periphery. In this material many of the double pachytene filaments show signs of entering the second contraction before segmentation has occurred. The author did not find satisfactory evidence of loops, the arms of which have been reported to twist about one another to form the pairs of chromosomes. The segments of the pachytene threads are long, extending across the nucleus. The number of these paired segments appears to be twelve.

The further history of chromosome reduction in *Lilium superbum* has been carefully followed, but, as there are no departures from generally accepted accounts, it will not be discussed in this paper.

#### DISCUSSION OF CONCLUSIONS

Although the reduction divisions have been studied and described for many forms with the object of determining the time, nature, and extent of the synaptic union of homologous chromosomes, there is still much disagreement concerning many details because of certain almost unavoidable flaws in the methods essential to the study of the process. The seriation of very early stages, so important to an understanding of synizesis, is particularly difficult because of lack of uniformity throughout an anther. Closely associated nuclei may show an advancing series of stages, or essentially the same stage. Not only may closely associated nuclei differ, but the chromatin throughout one nucleus may show several stages. Differences of opinion should be settled by direct evidence rather than by inference, but, since the process of chromosome organization cannot be observed in action, it is impossible to seriate stages by direct evidence.

To one unfamiliar with the material, it may seem a simple matter to count the number of segments produced by transverse division of the pachynema, but in sections 33 microns thick, which include a whole nucleus of *Lilium superbum*, the tangle of long segments presents a picture of confusion. Here too the worker is confronted with the difficulty of knowing just when segmentation has occurred. However, it is important for the theoretical and practical furtherance of genetics that the details of the reduction division be known; and it is only by the compiling of much evidence through exhaustive research by many observers that the complex process will finally be interpreted with confidence.

As previously stated, the theory of parasynapsis requires no regularity of alveolation and demands the resolution of the reticula into single, not double threads, and the pairing of these threads previous to synzesis. Segmentation of the double pachynema should then give, in the lily, twelve pairs of chromosomes initials. From the time of emergence of the thread system from synzesis to segmentation of the pachynema, there should be present evidence of its double nature. On the other hand, alveolation so regular as to give rise to a lengthwise split, and the resolution of the reticula into two threads, would give support to the telosynaptic interpretation. According to telosynapsis, there should be expected an approximation of the two threads and the ultimate disappearance of the lengthwise split before the association of chromosome segments has taken place. Also, segmentation in the lily should result in segments diploid in number, if the telosynaptic interpretation were correct.

The chromosomes of *Lilium superbum* characteristically show a single row of alveoli in the last archesporial telophase. The alveoli increase in number and become so irregularly distributed that the possibility of an initiation of a longitudinal split by the original series seems highly improbable. Furthermore, it is not possible to state with accuracy that the original series is absolutely axial in position. It is certain only that there is at first a single series or row of alveoli. It will be seen that these conclusions are in accord with those of Grégoire (1906), Sharp (1913, 1920b), and others, but are contrary to those of Fraser and Snell (1911), Digby (1919), Reed (1914), and Nothnagel (1916).

Sharp (1921, p. 231) states that

At the beginning of the heterotypic prophase the nuclear reticulum, without breaking down into such distinct elementary nets or alveolar units as are seen in the somatic prophase, takes the form of long slender threads (*leptotène* or *leptonema* stage).

I wish to emphasize that this stage should be sought in the very late resting nuclei and not in those entering or about to enter synzesis.

I have already pointed out in an earlier part of this paper the chief differences in actual observations between the schools of telosynapsis and parasynapsis. I need only recall that the finding of a single-thread stage in the early prophase of the heterotypic mitosis is vital to the parasynaptic interpretation. Such a stage can find no place in telosynapsis, except in such forms as show only single threads from synzesis to segmentation, as have been described by all students of *Oenothera* cytology, and also for *Lactuca* (Gates and Rees, 1921). *Lilium superbum* shows this single-thread stage with convincing clearness in the early prophases, but can not be placed in the *Oenothera* and *Lactuca* group since the doubleness of the threads during and following emergence from synzesis is so evident as to be generally recognized.

As previously stated, the seriation of stages is a significant factor in presenting the claims of a telosynaptic or parasynaptic interpretation. For

example, telosynapsis would not admit of a sequence of stages such as shown in figures 5, 6, 7, and 8 of this paper, or would place them following figure 11. In the latter case the two threads would be interpreted as due to a regular process of alveolation in the telophases of the last archesporial mitosis. But, as I have said, it seems impossible to seriate figures 5, 6, 8, and 11 differently because of the increasing thickness of the threads and their increasing sharpness of outline.

The figures of associating threads suggest two possibilities: (1) the threads may pair first at one end and gradually approach throughout their length, or (2) they may be attached at one end and pair through complicated loops or coils. It seems probable that parts of the thread system may become associated in one way and other parts in another. The exact method of pairing is a matter of lesser importance, since it is the time of pairing and not the method which is vital to the discussion.

Many observers have reported in various forms a fusion of threads during synizesis. A few, however, have observed that the paired threads can be found in all stages from their first association to the metaphase of the heterotypic mitosis (Grégoire, 1907, 1910; Montgomery, 1911; Wenrich, 1915, 1917). *Lilium superbum* presents evidence that the threads are paired during synizesis and in stages of emergence, and that this condition persists throughout pachynema. Moreover, the paired threads of pachynema have a high degree of independence such as would not be expected of the lengthwise halves of an univalent structure. The open loops and wide spaces which appear support the idea of independent and individual threads.

Free ends of the segmenting spireme appear first at the periphery in pairs. Sometimes a pair of chromosomes will segment out of the pachytene reticulum with both ends at the periphery, and such a pair does not become involved in the second contraction figure. More often, the position of a pair of chromosome segments is such that it is impossible to trace them throughout their length, because of the entanglement at the center of the nucleus. Only this much is certain, that the free ends appear first at the periphery, and that convincing evidence of loops was not found in this material. Moreover, the number of paired segments counted in thick sections, and verified through reconstructions from thin sections, was twelve, the haploid count for *Lilium superbum*. These conclusions support in these respects those of Allen (1905) for *Lilium canadense*.

It is perhaps fitting at this point to suggest that the early pairing of the threads described above presents abundant opportunities for the breaking and recombination of threads which is the cytological interpretation of the phenomenon of "crossing over" based on genetical evidence. Such forms as the lily, although presenting no obstacles to this interpretation of "crossing over," nevertheless, from the complex conditions of synizesis, contribute no positive evidence of importance. The suggestion of an interchange of material between homologous chromosomes given by Allen

(1905), following his description of a fusion of the associated threads (*i.e.*, that the units themselves do not fuse and that the splitting of the pachynema may not take place wholly along the fusion plane because of twists in the thread and for other reasons), seems unnecessary, since an actual fusion of the associated threads does not appear to take place in this material. Lastly, while *Lilium superbum* furnished some evidence of chromomeres in linear arrangement, the author did not find their appearance and numbers in her material sufficiently definite or their arrangement exact enough to warrant discussion.

#### SUMMARY OF OBSERVATIONS

1. Alveolation of the chromosomes of the last archesporial mitosis begins in the telophases.

2. The initial series of alveoli appears to be axial. Later alveoli are so placed that the chromosomes become resolved into irregular reticula with nothing that can properly be called a lengthwise split.

3. Pollen mother cells show a delimitation of unit reticula in very early prophase of the heterotypic mitosis.

4. The unit reticula become resolved into single zigzag threads in the manner described by Sharp for unit reticula of somatic nuclei.

5. Condensation throws portions of these single threads into tangled coils. The structure of the coils is often destroyed by poor fixation, and they become represented by flakes of chromatic material.

6. Pairing of the threads usually takes place before synzesis, but may be delayed until emergence. This pairing is parasynaptic.

7. In well fixed material the associated parasynaptic threads remain independent in all stages. Imperfect fixation may result in a fusion, more or less complete, of the associated threads.

8. The thread system emerges from synzesis as a double pachynema of parasynapsis, and this doubleness persists, in well fixed material, to the heterotypic metaphase.

9. The first breaks in the segmentation of the double pachynema occur at the periphery, and the segments are long, extending across the nucleus. Free ends appear in pairs.

10. Crosswise division of the double pachynema results in twelve segments, each of which develops into a pair of metaphase chromosomes, thus giving the diploid number of twenty-four chromosomes.

11. A segment of the double pachynema may be cut out at the periphery, and the pair of chromosome initials thus formed does not become entangled in the second contraction figure.

12. A lengthwise split develops in each of the associated chromosomes, but the exact time of its appearance has not yet been determined.

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### EXPLANATION OF FIGURES

Figures in the accompanying plates were drawn with the aid of a camera lucida, using a Spencer mono-objective binocular microscope no. 1-H, with Spencer 1.5 oil-immersion objective and 12 X eye-pieces. Magnification 1300 diameters, except for figure 17, which is 1700 diameters.

#### PLATE I

FIG. 1. Daughter nucleus following the last archesporial mitosis, showing late telophasic chromosomes with an axial series of alveoli.

FIGS. 2, 3. Later stages of daughter nuclei in the process of reconstruction. Chromosomes are undergoing further alveolation.

FIG. 4. Stage later than those of figures 2 and 3. Unit reticula unusually clear because of the absence of anastomoses.

FIG. 5. A pollen mother cell in the late resting stage or very early prophase. The connections between unit reticula are breaking down. In parts of the nucleus, condensation has thrown the reticulum into irregular zigzag threads.

FIG. 6. Early prophase. Condensation in a more advanced stage than that shown in figure 5. Threads are becoming less zigzag.

FIG. 7. Early leptoneuma. Parallelism of threads probably not due to pairing, but to chance association.

FIG. 8. Late leptonema. Condensation has thrown the threads at various points into tangled coils.

FIG. 9. Very late leptonema, showing the pairing of threads previous to synizesis. In several parts of the nucleus imperfect fixation has destroyed coils and gathered their material into flakes.

#### PLATE II

FIG. 10. A nucleus from the center of the anther showing poor fixation. The structure of the threads is destroyed, and the chromatin is drawn into irregular masses.

FIG. 11. Nucleus entering synizesis. Two examples of paired leptotène threads, twisted about each other.

FIG. 12. Mid-synizesis.

FIG. 13. Thin section of mid-synizesis showing the paired-thread system of parasynapsis.

FIG. 14. Spireme emerging from synizesis with evidence of paired threads.

FIG. 15. Spireme beginning to emerge from synizesis. Delayed association of one pair of threads may be seen at the right.

#### PLATE III

FIGS. 16, 16a. A single spireme, following emergence from synizesis. The width of the spireme suggests that the double threads are swollen and fused through imperfect fixation.

FIG. 17. Single spireme, following emergence from synizesis, given as an example of stages often interpreted as post-synizetic union of homologous paternal and maternal threads. In this case the single spireme is considered to be an example of fusion due to imperfect fixation ( $\times 1700$ ).

FIG. 18. Emergence from synizesis. Partial preservation of the paired threads is shown where the spireme appears double.

FIG. 19. Pachynema, corresponding to stages of figures 16, 16a, and 17 in time of appearance. Better fixation has here preserved the paired threads to give a double spireme.

FIG. 20. Pachynema. Fixation not as good as in figure 19, there being considerable fusion between the threads, but evidence of the double spireme is still present.

FIG. 21. Surface view of early second contraction, showing segments of the double spireme lying at the periphery of the nucleus. Segment *a* has both pairs of ends at the periphery. Such a segment probably would not take part in the second contraction figure.

#### PLATE IV

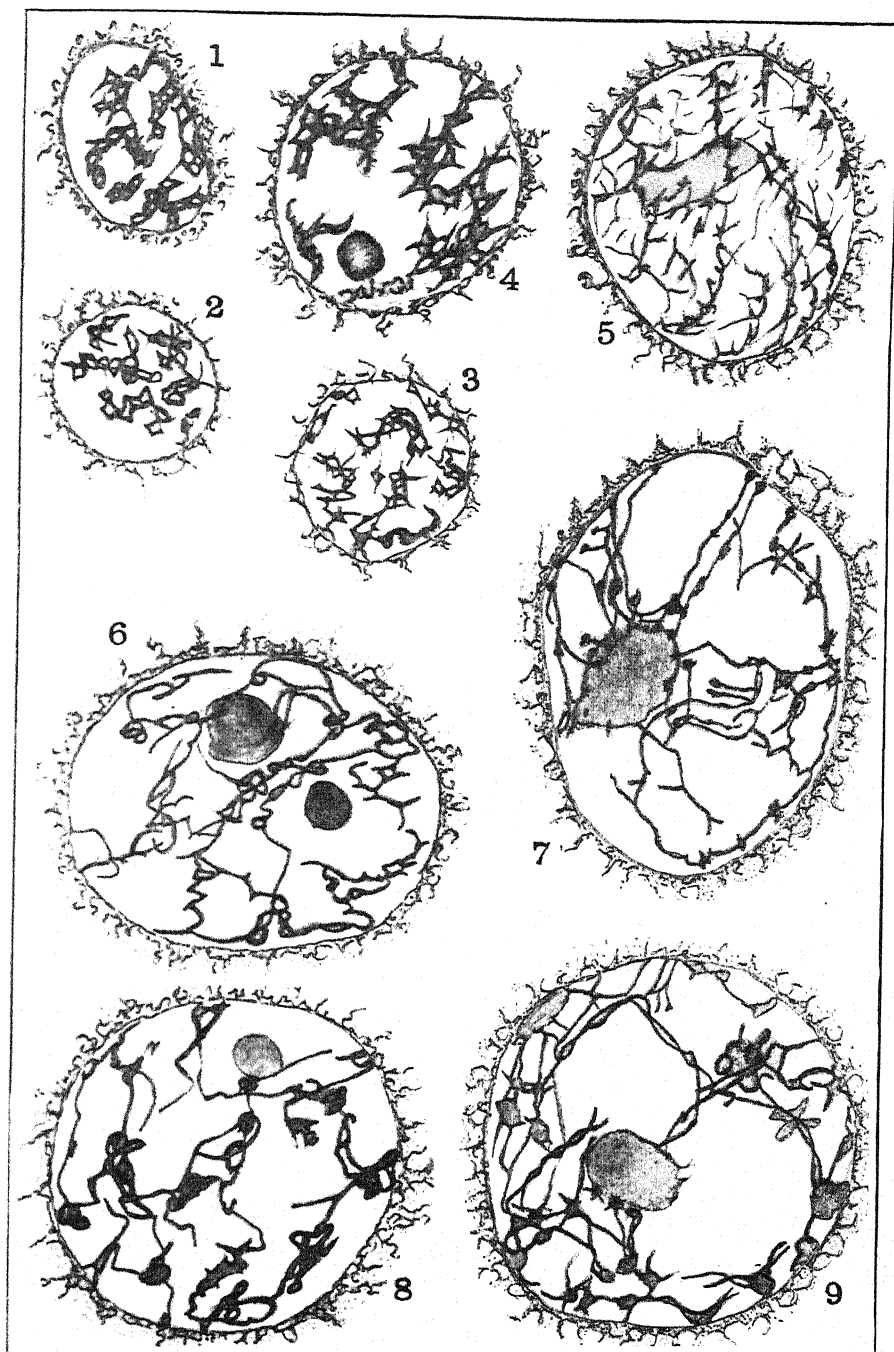
FIG. 22. Second contraction, somewhat later than the stage of figure 21. Long segments of the double spireme lying across the nucleus.

FIG. 23. Massing of the chromosome segments, leading to a typical second contraction figure.

FIG. 24. Second contraction. The pair of chromosome segments at the left have not become entangled in the second contraction figure.

FIG. 25. A pair of chromosomes, each showing a lengthwise split which is believed to initiate the division of the chromosome that takes place during anaphase of the heterotypic division in preparation for the homoeotypic mitosis.

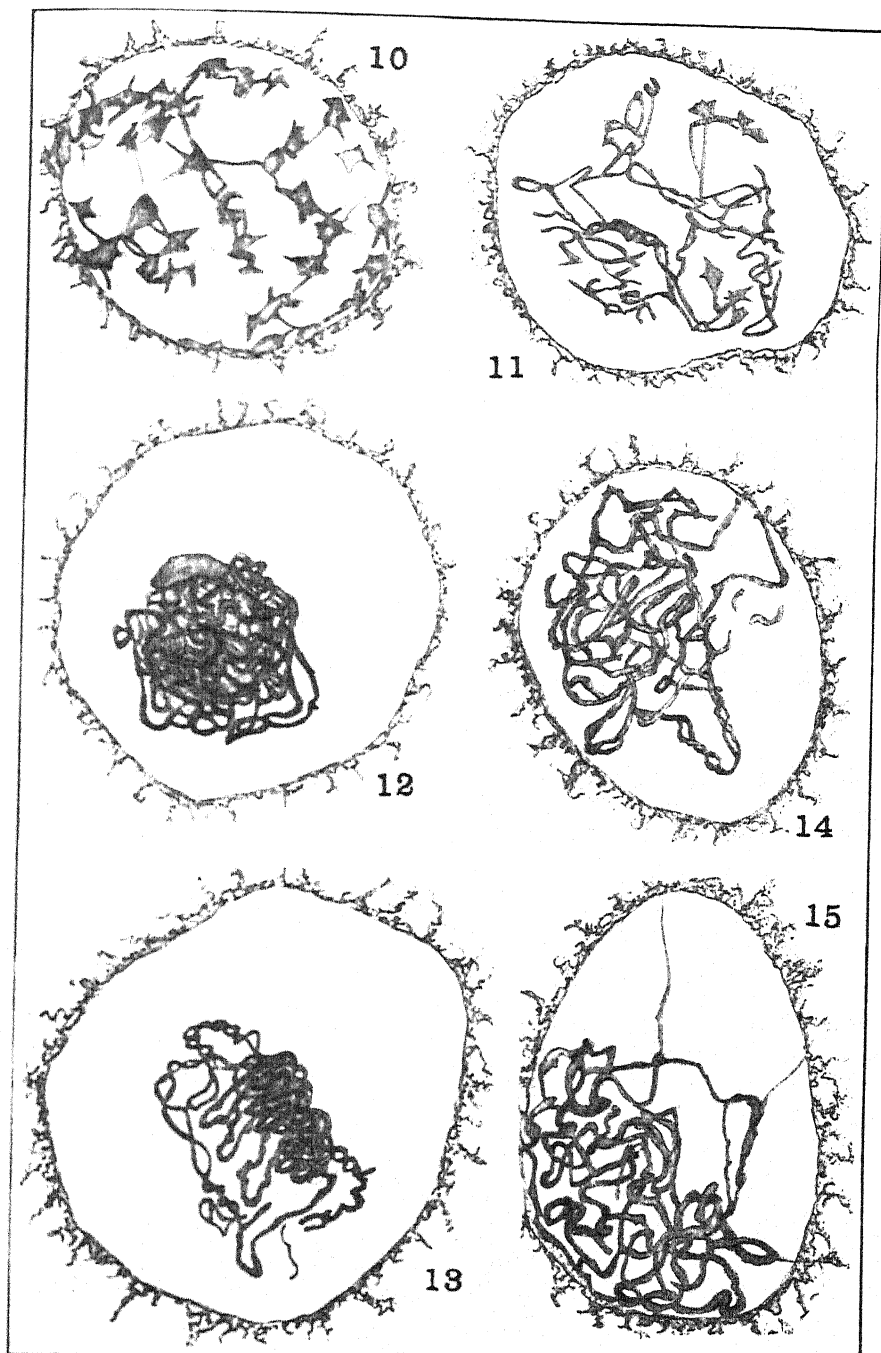
FIGS. 26, *a, b, c, d, e*. The most perfect loops presenting evidence for the telosynaptic interpretation offered by this material. All the figures were obtained from sections 15 microns in thickness, and therefore (since they do not include a whole nucleus) may show twisted chromosome segments cut at points of crossing which would then give the appearance of loops. Such fragments cannot be trusted to give reliable evidence of true conditions.



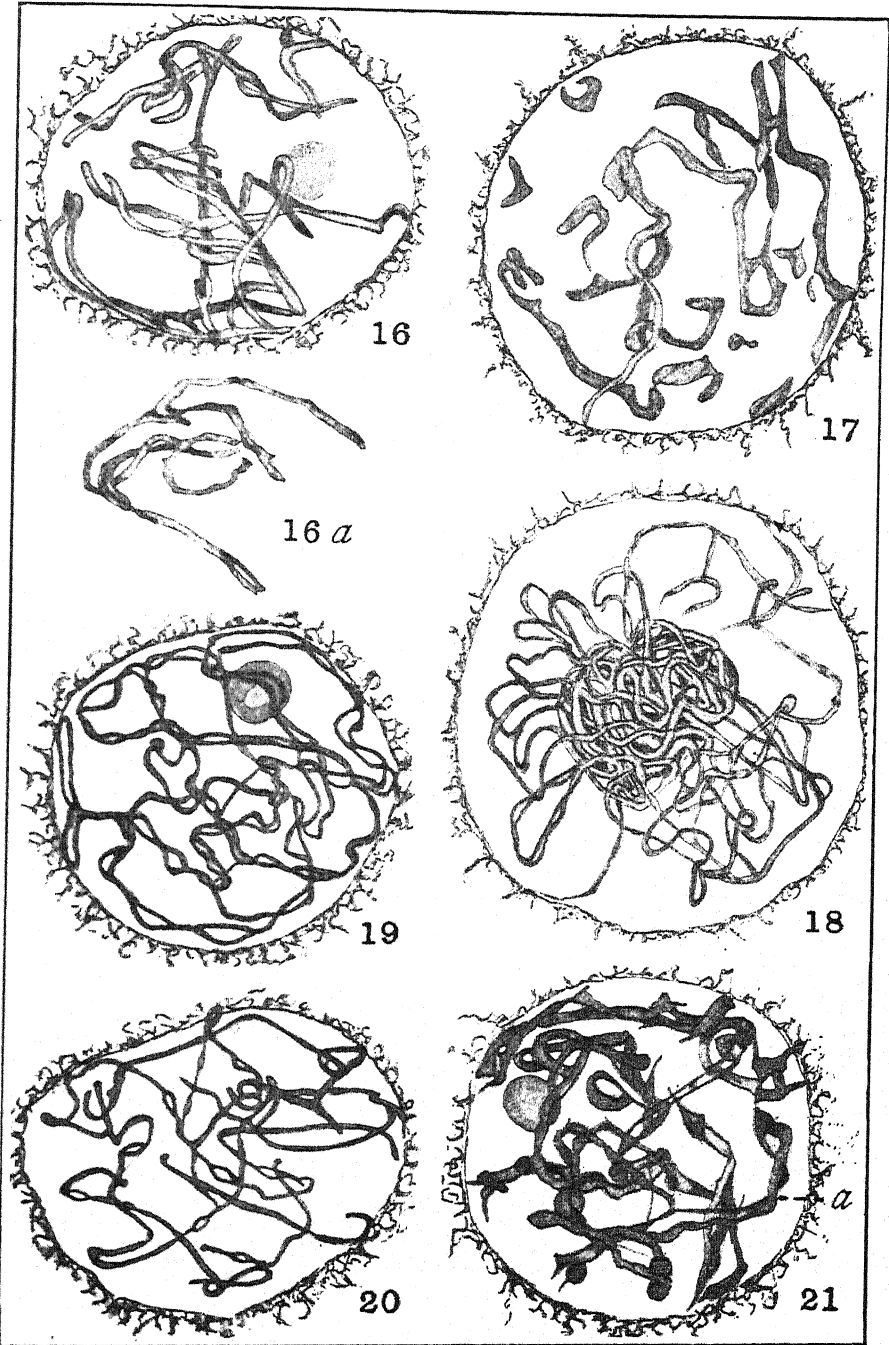
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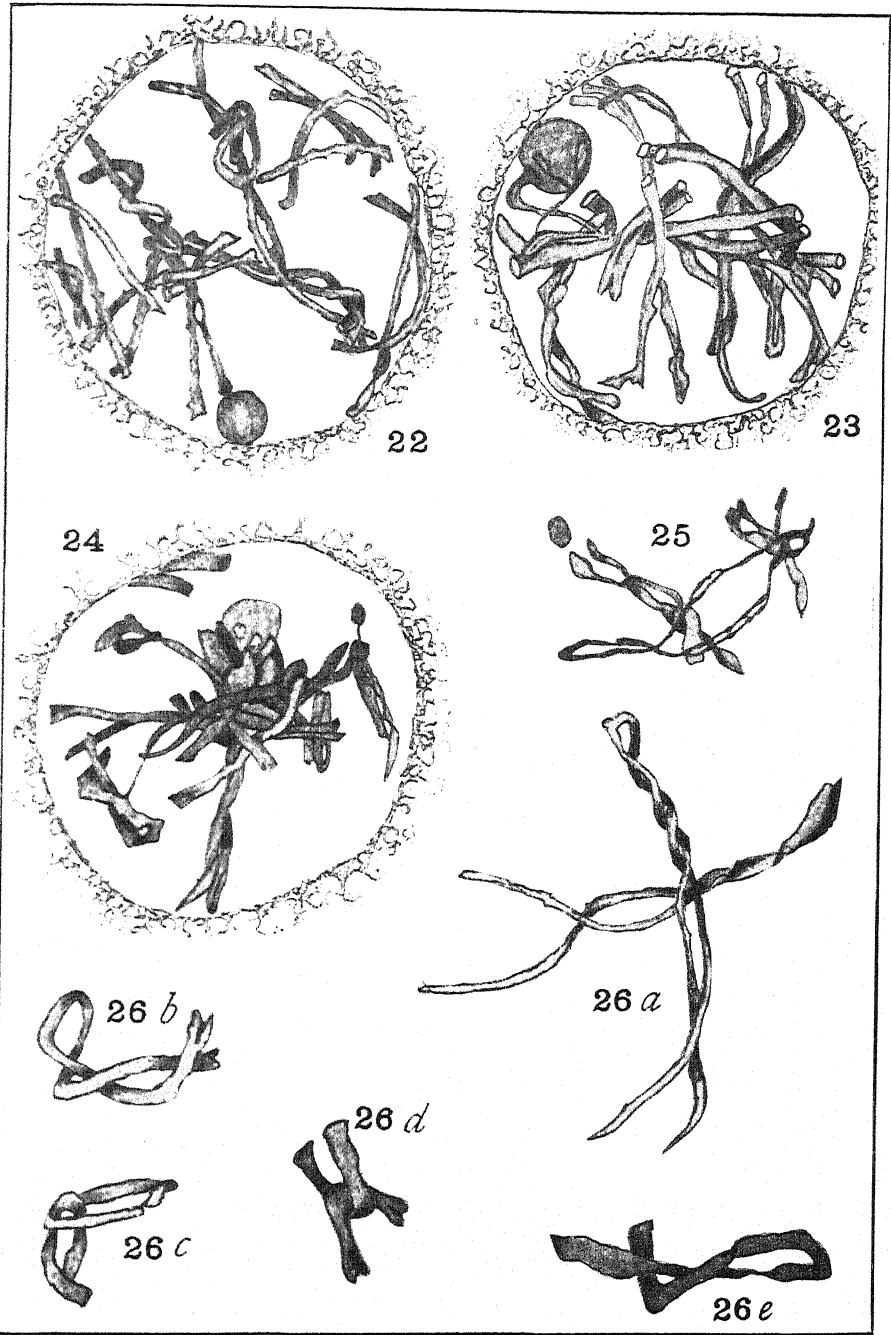






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# CYTOLOGICAL STUDIES OF THE MOSAIC DISEASE OF TOBACCO

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## INTRODUCTION

The recent revival of the theory that the mosaic diseases of plants are caused by microorganisms has aroused renewed interest in the study of the cell contents of mosaic plants. Such a study would be expected to show whether or not any visible microorganisms are associated with the disease.

Several workers have made cytological studies of the mosaic disease of tobacco as well as of other plants, but none of these studies seems to have exhausted the possibilities in this line of investigation.

Iwanowski (1) made a cytological study of mosaic tobacco plants and apparently found vacuolate bodies similar to those described in this paper. Since his illustrations are diagrammatic and are lacking in descriptive matter, it is difficult to determine how much detailed structure he was able to see in the bodies observed.

During the years intervening between the work of Iwanowski and that of recent years, numerous investigators reported having made histological studies of the mosaic disease of tobacco but apparently none found abnormal cell inclusions in diseased plants.

Palm (3) recently followed up the work of Iwanowski, using more modern cytological methods. He reports having found vacuolate bodies and small dark-staining bodies in cells of the "diseased" tissue of mosaic tobacco leaves. No illustrations are presented, and from the description one can not obtain a clear conception of the appearance of the cell inclusions which he describes. Foreign bodies have also been described by recent investigators in mosaic or mosaic-like diseases of other plants, but their significance is also questionable.

Johnson (2) has shown that the mottling of the leaves of tobacco plants inoculated with mosaic virus becomes most evident at a temperature of approximately 28° C., while mottling does not develop or is greatly inhibited at temperatures below 18° or above 34° C.

With the results of the above-mentioned workers as a background, it seemed to the writers that the cytological study of healthy and mosaic tobacco plants grown at different temperatures might give some evidence as to the relation which the bodies found in mosaic plants bear to mottling.

<sup>1</sup> Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.



It also seemed possible that other bodies which could be identified as products of the diseased leaf or as microorganisms might develop at different temperatures.

It was also thought that a cytological study of the progressive development of healthy and mosaic plants might show differences which had not been previously observed and which would give some indication as to the origin, nature, and developmental stages of the intracellular bodies observed by earlier workers.

## OBSERVATIONS

### Series I

The first work done was in the nature of a general study. Slides were prepared from the leaves, pistils, and anthers of mosaic plants grown in the field and in the greenhouse. Numerous fixing fluids were tried in an attempt to find one suitable for this work. Zenker's, Bouin's, the three Flemming's, chrom-acetic, hot Schaudinn's, and formalin-alcohol-acetic were among those tried. The latter consisted of 100 cc. of 50 percent ethyl alcohol,  $6\frac{1}{2}$  cc. of formalin, and  $2\frac{1}{2}$  cc. of glacial acetic acid. This fixative gave much the best results of any of those tried and was used in most of the later work. Exceptions to this general statement will be noted in the descriptions.

As a result of a study of the chlorotic tissues of 19 leaves, each from a different plant, bodies of a spherical or ovoid vacuolate type (Pl. V, fig. 7, A) were found in 8 of these leaves. Such bodies were usually accompanied by a striate secretion-like substance. Both of these types of cell inclusion will be described in detail later. The fact that so few of the leaves contained these bodies is rather perplexing, since later studies have shown them to be almost always present in mottled mosaic plants grown in the greenhouse. It is very possible that other portions of these leaves may have contained the bodies, our examinations having been of small pieces about 0.5 cm. square. It is interesting to note that, in the first experiment, of the pieces from 10 leaves known to have come from plants grown in the field only 2 showed the presence of the bodies in question, while of those of the 5 leaves known to have grown in the greenhouse 4 contained such bodies. All the later work was done on plants grown in the greenhouse, so it is still questionable whether the vacuolate bodies are generally found in the chlorotic tissues of the leaves of mosaic plants grown in the field.

An examination was made of the non-chlorotic tissues of 19 mottled mosaic leaves, each leaf being from a different plant. None of the non-chlorotic tissues contained the vacuolate bodies or the striate material.

Only two healthy leaves were examined in this experiment, and, as in later experiments, none of the various types of abnormal cell inclusion were found in the leaves of healthy plants.

Five pistils and seven anthers, each from a different mosaic plant, were examined. Of this material, only one of the anthers contained any visible,

apparently abnormal structure. In several of the peripheral cells of one of the anthers, bodies were seen resembling the vacuolate bodies found in the chlorotic tissues of mottled leaves. However, these cells were so poorly fixed that the bodies in question could not be identified with certainty. Some of the same tissues were fixed in a solution composed of 100 cc. of 70 percent ethyl alcohol and 2 cc. of formalin. This fixative gave fairly good results, but was not as satisfactory as the formalin-alcohol-acetic fixative described above. The excellent fixation of onion root tips obtained with Flemming's medium solution led the writers to try this fixative with leaves, anthers, and pistils of tobacco. The results were very unsatisfactory, and, while bodies were observed in the chlorotic tissues of the leaves, the structure was so indistinct that it seemed useless to attempt the further use of this fixative for this purpose.

### Series II

In this series of observations an attempt was made to determine whether the temperature at which the plants were grown showed any correlation with the development of intracellular bodies. As stated above, it has been shown that mottling is inhibited at temperatures above 34° C. and below 18° C. It seemed possible, therefore, that the development of intracellular bodies of the type found in the chlorotic tissues of mottled mosaic leaves might also be affected by these temperatures.

Accordingly, on November 6, 1922, potted tobacco plants were inoculated by pricking the leaves with needles that had been dipped in juice expressed from mosaic leaves. This method of inoculation has always given practically 100 percent infection. The plants were then placed in 3 chambers, one chamber being kept at 18° C., one at 28° C., and one at 34° C. These chambers have been described by Johnson (2), and ordinarily do not vary by more than one or two degrees.

At 5 P.M. on November 18, 1922, 12 days after inoculation, small pieces (about 1 cm. square) were cut from the leaves of the plants in the different chambers and were placed in the alcohol-formalin-acetic fixative. After leaving them in the fixative for 48 hours, the pieces were run through the alcohols, chloroform, and paraffin. The tissues from the plants grown at different temperatures and from leaves of different sizes were kept in different lots.

#### *Plants Grown at 18° C.*

None of the plants grown at this temperature showed any visible mottling.

*Lot 50.* This lot was made up of pieces from very young apical leaves  $\frac{1}{2}$  to 1 inch long. A piece was taken from each of 2 leaves from each plant. Preparations were made from 6 of these leaves. The tissues in this lot were very young, the chloroplasts being as yet undeveloped. The sections cut

from these pieces were very well fixed, but no abnormal intracellular bodies of any type were found.

*Lot 49.* The pieces in this lot were taken from leaves of the same plants as those from which lot 50 was obtained. The leaves used in this case were young upper leaves about 2 to 4 inches long. Of the 6 leaves sectioned, only one showed any visible abnormal cell inclusions. Figure 1 is a drawing of a palisade cell from this leaf. No visible mottling had developed in this leaf, but the abnormal cell inclusions were localized in the cells in certain portions of the leaves. As has been found by other workers, the palisade cells in chlorotic tissues are shorter and more nearly square in section than those in the non-chlorotic tissues of a mottled mosaic leaf or than those in healthy leaves. Thus in a section it is easy to distinguish the chlorotic from the non-chlorotic tissues, providing mottling has developed sufficiently. In this particular instance it can not be said that the small dark-staining granules or bodies are in the chlorotic parts, because these parts are not distinguishable from the non-chlorotic parts. This cell, however, contains the striate material which in mottled leaves has been found only in the chlorotic tissues. As will be seen in the drawing, the cells contain numerous very small bodies that stain black with Heidenhain's iron-alum haematoxylin or red with Flemming's triple stain. Many of these bodies stain more darkly at the ends. The bodies of this type often assume a clavate form, both ends tending to stain darkly. The larger end usually contains the larger dark-staining portion. As can be seen from the scale, these bodies are quite small, varying from 0.6 to 5 microns in length. While not always the case, it is usually found that a cell containing a number of these small bodies has a somewhat shrunken and apparently degenerated nucleus.

From the drawings it is evident that in this leaf the palisade cells and their chloroplasts are well developed. A leaf of this age will probably never become badly mottled. At least part of the chlorotic appearance in mottled leaves seems to be due to the failure of the palisade cells in the chlorotic part to elongate. It is a common field observation that, when old plants are inoculated with mosaic virus, the older leaves do not become mottled, only the young growing leaves developing this symptom.

#### *Plants Grown at 28° C.*

The upper leaves of the plants grown at this temperature were distinctly mottled at the time that the pieces were placed in the fixative.

*Lot 46.* The pieces fixed in this lot were taken from very young apical leaves from  $\frac{1}{2}$  to 1 inch long. These leaves were too young to show distinct mottling, being very pubescent and light green in color.

This lot was very well fixed and showed intracellular bodies of various forms. Of the 8 leaves sectioned, only 2, apparently the youngest, did not contain abnormal intracellular bodies. The first small black-staining bodies appeared in the cells along the edge of the leaf and in the glandular trichomes.

In very young leaves the bodies were often found only in these cells. Figure 2 shows a cell of one of the short glandular trichomes, containing cell inclusions of two types which are not found in healthy leaves.

The striate or corrugated material appears to be produced quite early in the development of the disease, and when it first appears it usually seems to be connected with, and to radiate from, the nucleus (figs. 2, 3). In later stages of the disease this striate material does not show this relation to the nucleus (figs. 4, 5, 6), but may be found in various regions of the cytoplasm. Numerous stains have been tried in this work, the most successful being Heidenhain's iron-alum haematoxylin used alone or followed by orange G. The magenta-picro-carmin stain used by Calkins in staining the intracellular bodies associated with variola, and the eosin-methylene-blue combination used by Mallory in the study of the intracellular bodies associated with scarlet fever, were also tried. Neither of these stains gave good results in tobacco tissues. Flemming's triple stain was used considerably, and for certain purposes was found very satisfactory. The striate material has never shown much affinity for any of these stains. Its structure was best brought out by Heidenhain's haematoxylin. This stain gives the striate material a yellow color similar to that taken by the cell walls.

The other type of abnormal cell inclusion shown in figure 2 is the small body seen at A. Bodies of this type are common in the cells of young apical leaves. Usually a cell contains only one or two of these bodies, which, while sometimes in contact with the striate material, often show no relation to it. These small bodies stain a deep black with haematoxylin, but vary considerably in their staining reaction toward Flemming's triple stain, having been observed in colors varying from red through salmon pink and yellow to slightly violet. In form they vary from spherical to clavate. While this type of body seems to be of general occurrence in the cells of short glandular trichomes during the early stages of mottling, it does not appear to be as common in the epidermal, palisade, and mesophyll cells. However, bodies of this type have been found in many of these cells, and whether they represent a stage in the development of some of the larger types seen in figures 3, 4, 5, 6, 7, and 10 is still an open question. In the later stages of mottling, the cells in the chlorotic portion of the leaf usually contain ovoid or spherical vacuolate bodies. Such cells also contain the striate material, but never have been observed to contain the small bodies of the type shown in figure 2. These observations, and the fact that the vacuolate and the smaller bodies show a similar staining reaction, furnish some evidence that these small bodies may be an early stage of the vacuolate bodies. Small bodies of this type were also seen in leaf cells containing mitotic figures.

In figure 8 is shown a body adjacent to the nucleus in an epidermal cell. This body was very clear-cut, the dark-staining portions in the ends, and the very minute, dark-staining granules connected by a strand, being very distinct. Since this is the only body which was observed to show this

detailed structure, we do not at this time feel that it should be considered of much significance. It is interesting, however, to note that its apparent structure is somewhat similar to that of some of the protozoa. This body is very small and requires a high magnification to bring out its detailed structure.

A type of body commonly found in the cells of the short glandular trichomes of this lot is seen in figures 5 and 10 at *A*. A body of this type contains a dark-staining central granule which is usually adjacent to small vacuoles. These bodies are granular in structure, are often near the nucleus, and in the trichomes their appearance often precedes that of the large vacuolate bodies of the type shown at *A* in figure 7.

Crescent-shaped vacuolate bodies of the type seen at *A* in figures 3 and 4 were quite prevalent in some of the leaves of this lot. These bodies, in internal structure and staining reaction, resemble the large, rounded vacuolate bodies to be described later, and were in nearly all cases adjacent to the nucleus.

*Lot 45.* The pieces in this lot were taken from larger leaves from the same plants that supplied lot 46. The leaves used in lot 45 were from 2 to 4 inches long and were visibly mottled. Each of the 6 leaves sectioned contained abnormal intracellular bodies. While the mottling of these leaves was distinct enough to be visible when the leaves were attached to the plant, it had not developed sufficiently to be evident in the sections.

As might be expected, bodies of some of the types found in lot 46 were also found in lot 45, but the vacuolate forms seen in figures 3, 4, and 7 predominated in the latter lot.

Bodies of the large spherical or ovoid vacuolate type shown in figure 7, at *A*, were the most common in this lot. Bodies of this type vary in size from one half to several times the size of the nucleus. When found in the large, long cells of the long trichomes, these bodies are often of very unusual forms, some being very long and narrow while others are irregular in shape. The most common form, however, is that shown in figure 7, *A*. These bodies often lie near the nucleus, but are about as frequently found in the peripheral region of the cell.

The vacuolate bodies found in the cells of the short glandular trichomes are granular in structure, but those occurring in other cells of the leaf are quite homogeneous. The cytoplasm in the cells of the short glandular trichomes seems likewise quite granular as compared with that in the other cells of the leaf, and it is interesting to note that the structure of these bodies shows a similar relation.

When stained with a certain combination of Heidenhain's haematoxylin, the parts of a vacuolate body immediately about the vacuoles are slightly darker than the rest of the body. The bodies bear little resemblance to the nuclei or plastids of the leaf cell. With the triple stain, the vacuolate bodies usually appear pink while the nuclei and plastids of the leaf cells are blue.

With haematoxylin the bodies in question are usually a deep black, and, if stained for a period of several hours or more, the stain can nearly all be taken out of the nuclei and plastids while the vacuolate bodies remain deeply stained. The internal structure of the bodies is also quite different from that of the nuclei or of the plastids. The bodies usually have a dense homogeneous structure, the nucleus and plastids being reticulate and granular.

As seen at *A*, figure 6, the vacuoles sometimes contain dark-staining material, suggesting a structure found in the nuclei of some amoebae. This leaf cell also contains 4 very small rectangular bodies. Bodies of this type were found in both healthy and diseased leaves. They stain an intense black with Heidenhain's haematoxylin and a bright red with the triple stain. In form they resemble crystals, but their staining reactions do not resemble those of any known crystals.

Figure 11 represents a body of a very peculiar type. This body is in most respects very similar to those of the ordinary vacuolate type. However, it contains a distinct dark-staining smaller body which seems to be divided into two similar parts. The structure of this smaller body was very distinct and clear-cut, but, since this is the only instance in which such a structure was found in a vacuolate body, it can not at present be considered as of great significance.

Pieces were fixed from the very young apical leaves from these same plants on November 25. Of the eight leaves sectioned, 2 contained a very few of the small black-staining bodies of the type shown at *A* in figure 2. Some of the cells of one of these leaves contained some of the striate material. The other leaves of this lot showed no abnormalities. These observations are of interest in that they indicate that, when a given minimum period has passed after inoculation, the type of abnormal body found in a leaf depends on the state of development of the leaf rather than on the period since inoculation. The fact that we find different types of bodies in leaves of different ages and in cells of different tissues in a single plant may have some significance. This condition might be expected if these bodies were products of the disease, but would seem less likely to occur if they were stages in the cycle of an organism.

We find the striate material in the same cells with bodies of all the types described. In mottled mosaic leaves the striate material is found only in the chlorotic tissues, and, since it is found in the same cells with bodies of all the above-described types, it seems probable that bodies of all these types occur only in chlorotic tissues or in tissues which, if young enough, will become chlorotic.

#### *Plants Grown at 34° C.*

No visible mottling developed in any of the plants grown at this temperature.

*Lot 48.* The pieces fixed in this lot were taken from very small apical

leaves varying from  $\frac{1}{2}$  to 1 inch in length. Of the 6 leaves sectioned, 3 contained some of the striate material, and 1 contained a few bodies of the small type shown at A, figure 2.

*Lot 47.* The pieces fixed in this lot were taken from young upper leaves from 2 to 4 inches long. Pieces from 6 leaves were sectioned, and the only apparently abnormal cell inclusion found was in the form of a slight amount of striate material in the cells of one leaf.

The small numbers of bodies found in lots 47, 48, 49, and 50 indicate that the development of the intracellular bodies in question may be correlated with temperature as well as with mottling.

Other lots of tissues from these plants were later fixed. These tissues were subjected to a partial vacuum soon after being placed in the fixative. The resulting sections were very much distorted, apparently because of the high vacuum, and consequently most of this material was discarded.

### Series III

The observations above described showed the presence of various forms of abnormal cell inclusions in the leaves of mosaic-diseased plants. Even with all these bodies present, we were still unable to come to any conclusions as to their nature and their relation to one another. Therefore, it seemed advisable to fix tissues from developing plants at frequent intervals in an attempt to follow the development of these bodies.

Accordingly, 32 healthy potted tobacco plants were taken into a greenhouse which was set for a temperature of 29° C. These plants were all of about the same size, having an average height of about 6 inches. Twelve of the plants were left uninoculated and were placed in one end of the greenhouse, and the 20 plants to be inoculated were placed in the other end of the same greenhouse. The inoculations were made January 31, 1923, at 9 A.M., 1 P.M., 5 P.M., and 9 P.M., 5 of the 20 plants being inoculated at each hour mentioned. Because of other work it was impossible to fix material at intervals of much less than 12 to 24 hours, and it seemed that inoculation at frequent intervals might decrease the chances of missing some stages in the development of the bodies.

Beginning on February 3 at 5 P.M., pieces were fixed from the leaves of one of the plants inoculated at each of the 4-hour periods and from two of the uninoculated plants. The piece from each plant and each leaf was cut in a given form, *e.g.*, triangular, square, etc., so that it could be identified. Sterile instruments were used in taking pieces from the uninoculated plants in order to keep these plants free from mosaic. A sharp cork-borer about 1 cm. in diameter was used to cut the pieces from the leaves. This instrument made a clean cut and did not mutilate the remaining portion of the leaf. The piece to be fixed was then cut to the desired shape with a sharp safety-razor blade, and after being dipped in absolute alcohol for two or three seconds was placed in the fixative. Adjacent pieces of the same leaf

of each plant were cut and fixed, usually at 12-hour intervals. The first pieces were usually taken from the edge of the leaf so as to cause the least possible disturbance in the metabolism of the remaining portion of the leaf. Records were kept of the length of the leaves from which pieces were cut and of the degree of mottling shown by the leaves. As the disease developed and the leaves of the first series grew larger, new series were started on younger leaves of both healthy and mosaic-diseased plants. The first series was continued until February 15, 1923. The last pieces were taken from these plants on February 26, 1923. All the pieces were fixed in the alcohol-formalin-acetic fixative already described.

As soon as this material was examined, it became evident that the preparations were not nearly as satisfactory as those obtained in series II. The cause of this difference has not yet been determined. While much of the material has not been cut because it gave poor preparations, some of the lots have been sectioned and stained, and the observations made on these will be given.

The results of observations made on a series of sections taken from a single leaf at 12- to 24-hour intervals will first be discussed. The first abnormal intracellular bodies observed were of the ovoid vacuolate and the striate types. They were found in this leaf 6 days after inoculation. Bodies of these two types occurred in the same cells in certain localized portions of the leaf. At this time the leaf was  $3\frac{1}{2}$  inches long, but no mottling could be seen either in the leaf attached to the plant or in the pieces cut. A few cells in these sections contained numerous small bodies of the type shown in figure 1. These small bodies have never been found in the same cells with the large vacuolate bodies. When present, bodies of either type are usually found in practically all the cells of certain parts of the leaf. Sometimes the groups of cells containing the bodies of the two types are adjacent, and in other cases they are separated by cells which contain no abnormal intracellular bodies. The striate material was found in the cells containing bodies of each type. The same condition was found in the portions of leaves which were fixed 7,  $7\frac{1}{2}$ , and 8 days after inoculation. Mottling was first observed on this plant 8 days after inoculation. Only vacuolate bodies and striate material were observed in the portions fixed 9 days after inoculation, while those fixed  $9\frac{1}{2}$  days after inoculation contained the small and the vacuolate bodies and the striate structures. The 10-day portions showed only the vacuolate bodies and the striate material, while those fixed after 11, 12, and 13 days contained all three types. Only vacuolate bodies and striate material were found in the 14-day portions; structures of all three types were present in the 15-day portions. The presence of bodies of either of the two or the three types was observed until the 26th day after inoculation, when the last tissue was fixed. The same irregularity was found in another leaf, the first bodies in this case being found four days after inoculation and consisting of striate material, vacuolate bodies, and small black-staining bodies.



Striate bodies and ovoid vacuolate bodies were found in a small apical leaf fixed 8 days after inoculation, when the first tissue from this leaf was fixed. The same types of bodies were found in this leaf 9 and  $9\frac{1}{2}$  days after inoculation.

It is interesting to note that in the cases in which the small bodies were present in a cell, the nucleus of that cell was often apparently somewhat degenerated. In this connection it should be added that mitotic figures have never been observed in cells showing this type of abnormality. This, however, may be due to the fact that this type of abnormality has never been found in very young leaves.

From the above-described results it is evident that it is not an easy matter to determine the cycle of development of the various types of bodies, because we may find a cell containing either a large number of small bodies or one or two of the large vacuolate bodies at either an early or a later stage in the development of the disease. The fact that both types of bodies are found at various stages in the development of the disease indicates that both may persist for a rather long time, and it seems doubtful whether one is a developmental form of the other.

A short progressive series of sections was made of tissues from two other leaves. No bodies had appeared in these leaves 7 and 8 days after inoculation, when the last sections were cut. Sections were also cut from the portions taken from the leaves of three of the healthy plants. None of these sections contained any visible abnormal intracellular bodies.

#### Series IV

Since the formalin-alcohol-acetic fixative had given such good results in series I and II, it seemed that some modifications of this fixative might be even better. Portions of leaves were fixed in this fixative immediately after its preparation and after it had stood for three days. In the latter case, part of the tissues were dehydrated with alcohol and part with acetone. Other workers have found that dehydration with acetone gives very good results in dehydrating animal tissues containing Negri bodies. Another fixative, consisting of 100 cc. of 50-percent acetone,  $6\frac{1}{2}$  cc. of formalin, and  $2\frac{1}{2}$  cc. of glacial acetic acid, was tried. After fixing in this solution for 48 hours, the tissues were gradually dehydrated with acetone.

A fixative consisting of 100 cc. of water,  $6\frac{1}{2}$  cc. of formalin, and  $2\frac{1}{2}$  cc. of glacial acetic acid was also used. Part of this tissue was dehydrated with alcohol and part with acetone.

Part of the tissue fixed in the formalin-alcohol-acetic solution was fixed for only 24 hours, rather than for the 48-hour period formerly used.

Sections were cut from the various lots, and of all the lots those fixed in freshly prepared formalin-alcohol-acetic and in water-formalin-acetic seemed to be in the best condition. Fixation for 48 hours and the use of alcohol as a dehydrating agent seemed to give the best results. It is proba-

ble that both these fixatives might be improved by various modifications, such as using them while warm or modifying the length of time that the tissue is left in the fixative.

### Series V

The preparations obtained in series III were so unsatisfactory that it seemed advisable to repeat this work. We were particularly anxious to determine the early stages of the development of the abnormal intracellular bodies, since these might give us evidence as to their origin.

Accordingly, 4 potted plants, each about 6 inches in height, were inoculated at 4 P.M. April 19, 1923, 4 uninoculated plants being kept as checks. The inoculated and uninoculated plants were kept at opposite ends of the same greenhouse, the house being kept within a temperature range favorable for the development of mottling.

The first portions were cut from the leaves on April 23, at 11 A.M. In this series no attempt was made to keep the tissues from each leaf distinguishable, since it was thought that poor preparations obtained in series III may have been due to mutilation while cutting the leaf pieces into various forms. The great variation we had found in the types of bodies in the adjacent parts of a single leaf indicated also that average results from a number of leaves might be as valuable as the results from single leaves. Pieces were cut from the various leaves at intervals of 12 to 24 hours, the pieces from leaves of approximately the same size being kept together.

The first mottling was observed on these plants April 26 (7 days after inoculation), and in this experiment the first bodies were observed in portions cut and fixed on this same day. Sections were cut from pieces of four medium-sized leaves ( $2\frac{1}{2}$  to 4 inches in length) taken at this date. Each leaf was from a different plant.

One of these leaves contained no visible abnormal intracellular bodies, one contained only a few of the spherical or ovoid vacuolate bodies of the type shown in figure 7, A, another contained both vacuolate bodies and striate material, and the fourth contained striate material and small vacuolate bodies, most of the latter being of the small crescent type shown in figures 3 and 4.

Of the pieces taken from large leaves (5 to 7 inches in length) on this date, those from all three of the leaves contained striate material and vacuolate bodies of the type shown at A, figure 7.

Sections were made of portions of four very young leaves 1 to 2 inches long, each leaf being from a different plant. These were fixed on the same date as those described above. Vacuolate bodies and striate material were found in all these leaves. In two of them the small crescent form of body adjacent to the nucleus was the prevailing type. Figure 3 is a drawing of a young palisade cell from this tissue. In one of the leaves abnormal bodies were found in many of the cells containing mitotic figures. Figure 4 is a drawing of a cell from this tissue. As seen in the drawing, the crescent-

shaped intracellular body (A) apparently did not change its form during mitosis. Since the vacuolate bodies are usually spherical or ovoid when not adjacent to the nucleus, they might be expected to round up during the division of the nucleus of the leaf cell.

So far no evidence has been found to show that these bodies divide during the division of the leaf cells. The entire body appears to be retained in one or the other of the daughter cells.

The fact that these crescent-shaped vacuolate bodies adjacent to the nucleus may be found in sizes varying from those just visible up to sizes as large as the spherical type of vacuolate body indicates that they may increase in size while adjacent to the nucleus. Since they are most prevalent in the early stages of mottling and have an internal structure like that of the spherical or ovoid type of vacuolate body, it appears likely that the crescent-shaped bodies may round up when they become detached from the nucleus, thus forming bodies of the spherical type.

Pieces from one leaf of each lot up to that fixed 10 days after inoculation were also sectioned. This series indicated a gradual transition, as the mottling developed, from the crescent-shaped bodies to those of the rounded vacuolate type seen in figure 7, A.

#### DISCUSSION

A careful study has thus far been made of approximately 345 slides, including various stages in the development of the mosaic disease, and, while we do not think that these observations warrant any final conclusions, we do feel warranted in giving an interpretation of the evidence as to the cycle of development of the peculiar intracellular bodies found in mosaic-diseased tobacco leaves.

The striate material, bodies of the small type (figure 2, A), and the crescent-shaped bodies seem to be the first visible abnormalities to develop. All these bodies seem to appear at about the same stage in the development of the disease.

The fact that the striate material is usually in contact with the nucleus during its early stages indicates that it may bear some rather close relation to the nucleus. The amorphous nature of this material indicates that it may be a product of the diseased cell or of the causal agent. This material is found in the same cells with bodies of all the abnormal types that have been observed, and apparently persists in these cells throughout the life of the leaf. The fact that leaves which are distinctly mottled contain the striate material in only the cells of the chlorotic tissue indicates that the bodies of all the abnormal types observed are in tissues which, if young enough, will become chlorotic. It is hoped that microchemical tests may later show the chemical nature of the bodies of all the types found. Such tests should give some evidence as to whether these bodies are cell products or are varied forms assumed by an invading organism.

In the younger leaves, one or two of the small bodies (fig. 2, A) are often found in each cell. While sometimes near the nucleus, these bodies are most often in the peripheral cytoplasm or in the striate material. The facts that this type of body has been found only in the small apical leaves, and that these bodies stain similarly to the vacuolate types, afford some evidence that this form may represent a stage in the development of the bodies seen in figures 3, 4, 5, 6, 7, and 10. This, however, certainly does not seem always to be the case. In many cases, particularly in the older leaves, bodies of the small crescent-shaped type (fig. 3, A) adjacent to the nucleus were the first to appear after inoculation. In the trichomes, the bodies of the types seen in figures 5 and 10 sometimes appear to occur before those of the large vacuolate type (fig. 7, A). In most cases, particularly in the other cells of the leaf, the vacuolate crescent-shaped bodies adjacent to the nucleus appear to develop into those of the large, rounded vacuolate type (fig. 7). So far as we have been able to observe, the large, rounded vacuolate bodies together with the striate structures seem to persist in the chlorotic tissue throughout the life of the leaf.

One of the most puzzling forms is that of the bodies shown in figure 1. There are usually a number of these bodies in a cell. In structure and staining reactions they are often very similar to bodies of the type shown in figure 2, A. However, there are usually only one or two of the latter in each cell, and they have been found only in small apical leaves during the early stages of mottling, while there are usually a number of the former type in each cell, and this type has been found only in the older leaves. The fact that bodies of this form have been found during various stages of mottling is some evidence that they may persist throughout the life of the mosaic plant rather than representing a transition stage.

With the exception of the striate material, all these types of abnormal intracellular bodies have shown a striking similarity in their staining reactions. While such similarity can not be considered proof of likeness in chemical nature, it is evidence of some value in that connection.

With the exception of the type of abnormality seen in figure 1, bodies of all the types described have been found in leaf cells containing mitotic figures. This fact is interesting in that it indicates that the presence of these bodies does not prevent what appears to be normal nuclear and cell division.

In conclusion, we wish to express our appreciation of the advice given us by Drs. C. E. Allen, Sophia Eckerson, and E. J. Kraus, and other members of the staff of the University of Wisconsin. The criticism and advice of Dr. Allen during all phases of the work have been particularly helpful.

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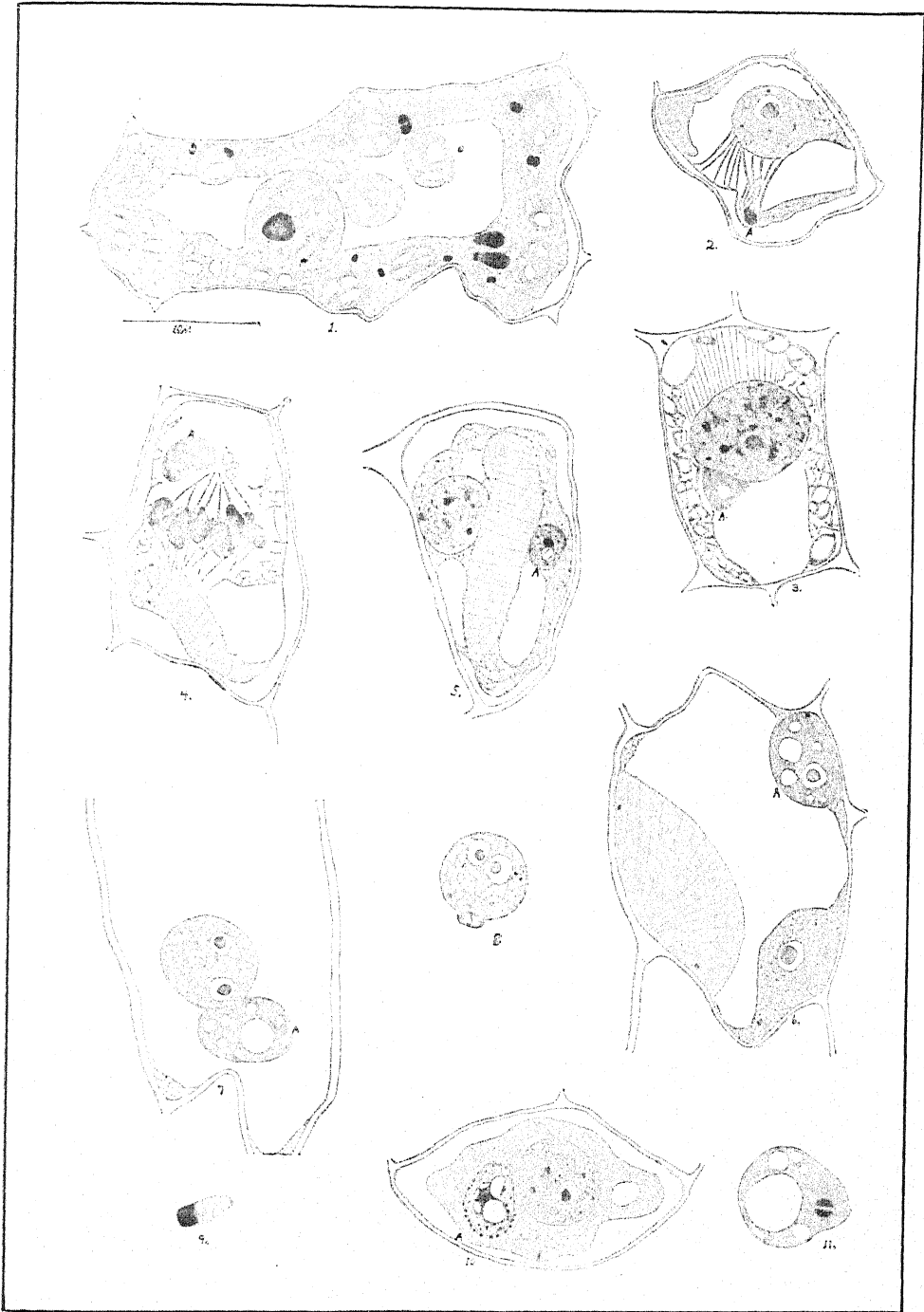
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2. Johnson, J. The relation of air temperatures to certain plant diseases. *Phytopath.* 11: 446-458. Pls. 21-23. 1921.
3. Palm, B. T. Is the mosaic disease of tobacco a chlamydozoonose? *Bul. Deli. Proefstation Medan, Sumatra* 15: 7-10. 1922.

## EXPLANATION OF PLATE

An Abbé camera lucida was used in outlining all drawings. A Zeiss apochromatic 2-mm. objective, N. A. 1.40, and an 18 x compensating ocular with a tube length of 145 mm. were used for the drawings. A 12 x compensating ocular was used in studying and drawing the details. The drawings were made to a magnification of about 3666 x and were reduced approximately one half in reproduction.

## PLATE V

- FIG. 1. Palisade cell containing a number of small, dark-staining bodies.
- FIG. 2. Cell of a short glandular trichome containing a small body at A, and striate material radiating from the nucleus.
- FIG. 3. Young palisade cell containing striate material radiating from the nucleus and a crescent-shaped body (A) adjacent to the nucleus.
- FIG. 4. Leaf cell containing mitotic figure, crescent-shaped body (A), and striate material.
- FIG. 5. Cell of a short glandular trichome showing striate material and vacuolate body (A), containing a dark-staining granule.
- FIG. 6. Epidermal cell containing striate material, small black rectangular crystal-like bodies, and vacuolate body (A) containing dark-staining material in one vacuole.
- FIG. 7. Epidermal cell containing the common vacuolate type of body (A).
- FIG. 8. Nucleus and small body from an epidermal cell.
- FIG. 9. A single body of the type seen in figure 1.
- FIG. 10. Cell of a short glandular trichome containing a body (A) of the same type shown in figure 5.
- FIG. 11. Peculiar type of body found in a palisade cell. Note the two similar dark-staining granules and the dark-staining material around the vacuoles.



RAWLINS AND JOHNSON: MOSAIC DISEASE OF TOBACCO



## A PRELIMINARY SKETCH OF THE PLANT REGIONS OF OREGON I. WESTERN OREGON.

MORTON E. PECK

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The collections upon which the ensuing discussion is largely based are mainly in the herbarium of Willamette University. Much use has also been made of material in the herbarium of the University of Oregon, and in that of the Oregon Agricultural College. The writer wishes to acknowledge his indebtedness to Professor A. R. Sweetser of the former institution, and to Doctor Helen Gilky of the latter for the privilege of studying these collections.

In order to realize how imperfect is our knowledge of the Oregon flora, one has but to glance through the botanical literature pertaining especially to the state. This consists of a few brief papers of a taxonomic nature, several local lists of species, some with short ecological discussions, and perhaps as many more of an economic character. There have also appeared four or five floras covering all or a part of Oregon in connection with other states. No attempt whatever has as yet been made to give even the briefest account of the plant distribution and the main factors of soil, climate, etc., concerned in it. The present paper is an undertaking of this sort, but, as the title indicates, it is a preliminary sketch only. With so little to depend upon save his own observations, the writer realizes that the results must be very unsatisfactory, but the attempt may perhaps serve to stimulate further research.

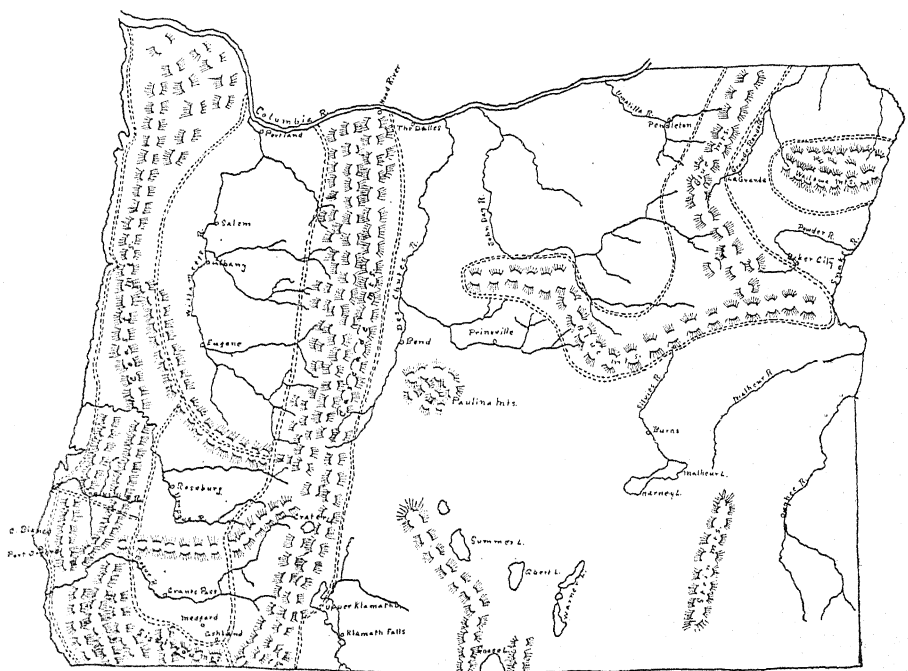
It is our purpose to give a very brief account of what appear to us to be the primary distributional areas of the state, with a general description of the vegetation of each area and the physical features that apparently determine it; and, finally, to add for each a list of those species that give it its characteristic floral aspect. The word "regions," as here used to designate the several sections into which our territory is divided, has been employed in so many different senses in works on distributional subjects that its use in this particular connection is scarcely desirable, yet no better term suggested itself. The names applied to these sections are still less satisfactory for the most part, and it is to be understood that all but one of them, namely, the Rogue-Umpqua, are but temporary makeshifts. Since the political boundaries of the state do not coincide with any physical barriers except on the west, the names of those regions that extend into adjoining states should of course be applicable to the entire territory



included; but, since the writer's investigations in this connection have extended little beyond the boundaries of Oregon, he has been wholly unable to suggest appropriate names, and has been obliged to adopt the clumsy alternative of employing names that are applicable to this state only. Of the nine regions suggested, only the one above mentioned lies wholly in Oregon. The most difficult and critical part of the undertaking has been the preparation of the lists of species. These must be as representative as possible and at the same time brief. In compiling lists of this sort, one may emphasize in his selection the endemic features of the flora of his territory, or he may assign more significance to the particular assemblage of forms and the relative abundance of individuals of the several species that go to make up the bulk of the total vegetation of the area, regardless of the extent of their range in other regions. The former course would be obviously impracticable in dealing with the flora of a single state whose boundaries for the most part do not constitute natural regional barriers. Furthermore, in an area no larger than that of Oregon we could scarcely expect any considerable number of species to be confined in their range to any one of the several sections proposed. At the same time, a particular assemblage of even widely distributed species may give to a limited region a strongly marked floral character; this is especially the case with forest vegetation. The lists, therefore, for the most part aim to include those species that are particularly abundant in the area in question, as well as those that are more or less limited to such territory in their distribution within the state. It is believed that by thus associating the most characteristic species with the most abundant, the lists may be made to show the more salient features, both quantitative and qualitative, of the flora. In dealing with a territory the details of whose plant distribution are so little known as is the case in Oregon, such lists must inevitably contain many errors. Numerous species that should be included in each of the lists have doubtless been overlooked, and some, though it is hoped a smaller number, might probably well be omitted. Unfortunately, the most valuable forms in this connection are those of the most limited distribution and consequently most likely to have been missed. In spite of these shortcomings, however, it is believed that each list is fairly representative of the territory covered. In the great majority of cases the writer has verified the records used, and all records considered doubtful have been rejected.

Oregon presents a remarkable diversity of conditions as to soil, moisture, and temperature. The annual precipitation ranges from 3250 mm. at points on the coast to 200 mm. in parts of Umatilla County, and the lowest winter temperature from barely freezing in southwest Curry County to arctic conditions on the highest mountains. The variety in soil composition is no less remarkable. For these reasons it has been found necessary to designate a larger number of plant regions than one might ordinarily expect to find in so limited an area.

The nine regions proposed are indicated on the accompanying map, though only in a general way, since in most cases sharply defined boundaries do not exist.



TEXT FIG. 1. Map showing the boundaries of the proposed plant regions of Oregon. Boundaries indicated by the double broken lines.

In the present paper we shall consider only the territory west of the Cascade Mountains. In a later paper the rest of the state will be discussed.

#### NORTHERN COAST REGION

This comprises the very narrow coastal strip from the mouth of the Columbia River to the mouth of the Coquille. It takes in also the salt marshes and flats about the several bays. On the landward side it passes more or less gradually into the adjoining region. In some sections, especially from Cape Perpetua to a little south of Hecata Head, it is largely eliminated by bold slopes reaching nearly or quite to the coast line. From the latter point southward most of the distance, the development of extensive sand dunes with their landward migration widens the region considerably but renders its boundary extremely vague, especially by giving rise to numerous lakes, ponds, and swamps which, though not quite fresh, are largely cut off from direct maritime influence.

The bays are mostly small and fringed with narrow tide marshes. The beaches are also narrow, and where they are interrupted by cliffs of basaltic

or other formation the latter have in places been cut away by wave action, leaving small steep islands just off shore. While dunes are most extensive toward the south, they occur here and there throughout the length of the region, usually having migrated inland until fixed by a permanent mantle of vegetation. Sphagnum bogs are numerous but not large.

The geological formation is varied, the northern two thirds consisting largely of Miocene sandstones and shales, the former extensively decomposed to loose sand. The prominent cliffs and headlands are mostly of Tertiary basalt. The southern third is mainly of gravel, sand, and silt of the Quaternary.

Considerable uniformity of moisture and temperature conditions, of course, characterizes this region. The lowest winter temperature at the northern end is never many degrees below freezing, and the warmest days in summer, even at the southern extremity, seldom exceed 70° F. The average annual precipitation in the north is about 2000 mm. and in the south 1500 mm., but there is great local variation. The average number of cloudy, foggy, or rainy days is about 280, and rainy days are common during July and August, the driest months. In winter, high winds from the sea, often amounting to very severe storms, are of almost daily occurrence. Similar though much milder winds prevail largely during the rest of the year.

Two principal factors determine the width of this region: one is the distance to which the direct maritime influence, that is, the strong salinity of the soil, extends inland, and the other, the force of the winds, which produce a powerful mechanical effect upon the vegetation. The particular contour of the land must of course also be taken into account, since it considerably affects both of these factors.

We find, as usual along sea coasts, a somewhat definite zonal distribution of the vegetation. This is most marked where the beaches are well developed and the rise to shoreward is gradual.

The lowest zone, covering the beaches, youngest dunes, and most of the seaward faces of shore cliffs and other steep slopes, comprises the more strongly halophytic species, mostly of low or prostrate habit, many of them of great importance in checking the landward drift of wind-blown sand. Most characteristic of these are the following: *Calamagrostis aleutica*, *Agrostis melaleuca*, *A. maritima*, *A. pallens*, *Poa macrantha*, *P. confinis*, *P. pachypholis*, *Festuca rubra*, *Elymus arenarius*, *Carex macrocephala*, *C. Lyngbyei*, *Scirpus riparius*, *Juncus Lescurii*, *J. falcatus*, *Rumex mexicanus*, *R. maritimus fueginus*, *R. persicarioides*, *Polygonum paronychia*, *Atriplex littoralis*, *Abronia latifolia*, *Spergularia marina*, *S. macrotheca scariosa*, *Stellaria humifusa oblongifolia*, *Sagina crassicaulis*, *Arenaria peploides*, *Pentacaena ramosissima*, *Cakile edentula*, *Lupinus littoralis*, *Lathyrus littoralis*, *L. maritimus*, *Conioselinum Gmelini*, *Lilaeopsis occidentalis*, *Glehnia littoralis*, *Statice arctica*, *Convolvulus Soldanella*, *Romanzoffia unalaschensis*, *Amsinckia lycopsoides*, *Orthocarpus castillioides*, *Plantago*

*Bigelovii*, *P. maritima*, *Agoseris apargioides*, *Franseria bipinnatifida*, *F. Chamissonis*, *Erigeron glaucus*, *Tanacetum huronense*.

Back of this more strictly maritime assemblage is a broader zone of a somewhat varied character, though fairly constant in several particulars. The high winds from the sea here constitute the dominant influence. The species, though not to be regarded as in any sense halophytic, are tolerant of a considerable degree of salinity. The area includes numerous old dunes, long, smooth seaward slopes, sandy knolls, brackish bogs and pools, and sphagnum bogs. There are extremely dense thickets of *Pinus contorta* and *Picea sitchensis* with various large shrubs. Lower thickets of *Gaultheria Shallon*, often absolutely impenetrable, occur nearly everywhere in somewhat protected situations. The more exposed slopes are largely covered with dense, rounded clumps of *Vaccinium ovatum*. To the eastward this area passes gradually into the heavy Coast Mountain forest.

Of the numerous species occurring in this zone, few are highly characteristic, that is, they are mostly of a wider distribution within the state than are those of the area last considered. The following list includes those that are especially characteristic as well as the most abundant: *Pinus contorta*, *Picea sitchensis*, *Agrostis oregonensis*, *Holcus lanatus*, *Bromus pacificus*, *Carex pansa*, *C. obnupta*, *C. sitchensis*, *Eriophorum Chamissonis*, *Juncus oreganus*, *Maianthemum bifolium dilatatum*, *Sisyrinchium californicum*, *Habenaria Michaeli*, *Salix Hookeriana*, *Myrica californica*, *Lepidium Menziesii*, *Gormaniana oregana*, *Boykenia elata*, *Chrysosplenium Scouleri*, *Rubus parviflorus*, *R. spectabilis*, *Sanguisorba microcephala*, *Potentilla anserina*, *Fragaria chiloensis*, *Lathyrus palustris*, *Hypericum anagalloides*, *Viola Langsdorfii*, *V. adunca*, *Sanicula Howellii*, *Angelica Hendersoni*, *Vaccinium ovatum*, *V. oxycoccus intermedium*, *Gaultheria Shallon*, *Arctostaphylos Uva-ursi*, *A. tomentosa*, *Ledum columbianum*, *Rhododendron californicum*, *Trientalis arctica*, *Castilleja miniata Dixonii*, *Galium cymosum*, *Hypochaeris radicata*, *Solidago glutinosa*, *Achillea millefolium* var., *Cirsium edule*.

Among the most characteristic species and those occurring in greatest abundance in the tide marshes about the bays are: *Triglochin maritima*, *Distichlis spicata*, *Puccinellia paupercula alaskana*, *Scirpus americanus*, *S. robustus*, *Juncus effusus hesperius*, *J. balticus*, *Salicornia pacifica*, *Glaux maritima*, *Cuscuta salina squamigera*, *Grindelia oregana*, *Jaumea carnosa*.

#### NORTHERN COAST MOUNTAIN REGION

This area includes both slopes of the Coast Mountains from the northern boundary of the state southward to the Coquille River. It is separated from the sea only by the narrow coastal strip. To the eastward it passes gradually into the Willamette and Umpqua valleys, and its southern boundary is no more sharply defined.

The mountains are low and rounded, the highest points reaching an altitude of 1200 to 1350 meters. Precipitous slopes and extensive rock

exposures are not abundant. The geological formation of the northern half consists mostly of Tertiary basalt and Oligocene shales and sandstones, while over the southern half sandstones and shales of Eocene age dominate. The soil, except in the narrow valleys, is mainly residual, heavy and clayey, often mixed with sand and gravel, mostly deep, and rich in humus.

The eastern slope of the Coast Mountains is more abrupt than the western, and the average temperature and moisture conditions differ markedly on the two sides. The western slope, being exposed to the damp, cool winds from the sea, has an annual precipitation of about 1875 mm. near the summit toward the northern boundary of the state, and moderately deep snows are common. Toward the southern end of the region, the precipitation at corresponding points is about 1625 mm. A little to the east of the summit of the range the precipitation falls to 1375 mm. in the north and somewhat less in the south, with much higher summer temperatures.

This region is one of almost continuous heavy coniferous woods, except where the latter have been depleted by lumbering operations and fire. Deciduous trees occur in the deep, narrow valleys of streams and on higher slopes that have been burned over. In general the eastern limit of the area is determined by the unbroken continuity of the forest. To the south the forest thins out somewhat and changes its character. The most important trees are *Pseudotsuga taxifolia* and, well down the western slope, *Picea sitchensis*; *Abies grandis* is also common, and *Thuja plicata* is abundant especially to the northward. *Alnus oregona* is the most abundant of the deciduous trees. Ferns and mosses comprise the most conspicuous elements of the herbaceous vegetation.

As a whole, the region is poor in species if we exclude those that have crept into the more or less cleared valleys from surrounding territory, and it is very poor in endemic forms; its character, however, is fairly well marked. The following list includes the most abundant species of this assemblage, together with a few peculiar to the area at least within the state: *Pteridium aquilinum pubescens*, *Struthiopteris spicata*, *Polystichum munitum*, *Selaginella struthioloides*, *Tsuga heterophylla*, *Abies grandis*, *Pseudotsuga taxifolia*, *Picea sitchensis*, *Deschampsia elongata*, *Trisetum cernuum*, *Lysichiton camtschaticense*, *Erythronium revolutum*, *Scoliopus Hallii*, *Disporum Smithii*, *Alnus oregona*, *Asarum caudatum*, *Ribes sanguineum*, *Tiarella unifoliata*, *T. trifoliata*, *Mitella ovalis*, *Holodiscus discolor*, *Pyrus diversifolia*, *Aruncus sylvester*, *Rubus laciniatus*, *Rubus macropetalus*, *Rubus spectabilis*, *Filipendula occidentalis*, *Oxalis oregana*, *Oxalis trilliifolia*, *Acer macrophyllum*, *A. circinatum*, *Viola sarmentosa*, *Epilobium angustifolium*, *Oenanthe sarmentosa*, *Vaccinium parvifolium*, *Cladanthamnus pyrolaeiflorus*, *Fraxinus oregana*, *Hydrophyllum tenuipes*, *Stachys pubens*, *Mimulus dentatus*, *Digitalis purpurea*, *Sambucus callicarpa*.

## WILLAMETTE VALLEY REGION

This section comprises the valley of the Willamette River. It borders the preceding region well down the eastern slopes of the Coast Mountains and is bounded by the Cascades to the eastward, extending along their bases to an altitude of approximately 300 meters. On the south it is separated from the Umpqua Valley by a low mountain ridge, the Calapooia Divide. None of these boundaries are very sharply defined. This is the most extensive tract of moderately level land in the state west of the Cascade Mountains.

The soil and topography present considerable diversity. The formation over most of the lower portion of the valley consists of Quaternary gravel, sand, and silt. This borders to the westward on sandstones and shales, partly of Eocene and partly of Miocene age, and to the eastward on the basaltic formation of the Cascades. Much of the lower land is very level, consisting of heavy alluvial soil rich in humus but poor in lime; there are also many gravelly areas. The floodplain of the Willamette is in most places very narrow, the soil being a mixture of river silt and fine sand. Large gravel bars are frequent. A number of bayous occur along the river, but lakes and swamps are few. The most noteworthy of the latter is Lake Labish, a shallow swampy lake a few miles north of Salem, which has now been drained but which once contained a remarkable assemblage of plants.

On either side, the valley rises to the adjoining mountains by a series of rounded hills occasionally with outcroppings of rock, but mostly covered with a fairly good layer of residual soil, which is clayey in composition, and, particularly on the west side, often mingled more or less with the more resistant fragments of the rock from whose decomposition it originated.

The average yearly precipitation for the whole Willamette Valley is about 1150 mm. Light snowfalls are not unusual, but the snow seldom remains for more than a week, and winter temperatures below 15° F. are rare. Summer temperatures are moderately high, and there is very little rain from the latter part of June till the middle of September. The latter part of the summer is thus very dry, so that over much of the territory the land barely escapes the necessity of irrigation for agricultural purposes.

The character of the vegetation of the Willamette Valley has been more extensively altered through human agency than that of any other part of the state. Most of the original forest has disappeared, while the invasion of the land by foreign species has largely displaced the native flora. The coniferous woods, however, were never dense and continuous like those of the Coast Mountains, and the trees were never of comparable size. *Pseudotsuga taxifolia* was the principal species, covering large areas, but these probably in all sections of the valley were interspersed with open slopes. *Abies grandis* was the only other coniferous species occurring in any considerable abundance; it was found in moist ground, especially along water

courses. The gravelly areas of the valley floor and many slopes too dry for *Pseudotsuga* bore a scattered or sometimes denser growth of *Quercus Garryana*. The flood-plains of the larger streams, particularly of the Willamette River, were dominated by deciduous trees—*Populus trichocarpa*, *Salix lasiandra lancifolia*, *Acer macrophyllum*, and *Fraxinus oregana*, while along smaller streams *Alnus oregona* and *Acer circinatum* were most abundant. The deciduous species have suffered less at the hand of man than the forests of *Pseudotsuga*, since they occupy land that is mostly unfit for agriculture.

The total number of shrubs and herbaceous species is very large, but there are comparatively few that are particularly characteristic of this area. The following list includes those forms that occur in the greatest abundance and that together make up the bulk of the vegetation, and a number of others which, though forming no considerable part of the total plant life, appear to have their center of abundance in this area. Native and introduced species must here be accorded the same treatment. The great abundance of the latter swells the list to rather inordinate length. They are marked in the list with an asterisk.

*Taxus brevifolia*, *Abies grandis*, *Pseudotsuga taxifolia*, \**Agrostis stolonifera*, *A. microphylla*, \**Holcus lanatus*, \**Arrhenatherum elatius*, \**Aira caryophyllea*, \**Avena fatua glabrata*, *Melica subulata*, *Bromus marginatus*, *B. carinatus*, *B. vulgaris*, \**B. hordeaceus*, \**Dactylis glomerata*, \**Poa annua*, \**P. pratensis*, *Festuca megalura*, \**Lolium multiflorum*, \**L. perenne*, *Elymus glaucus*, *Carex pachystachya*, *C. vicaria*, *C. Piperi*, *C. lanuginosa*, *C. exsiccata*, *C. Hendersoni*, *C. obnupta*, *Scirpus microcarpus*, *Juncus effusus pacificus*, *J. tenuis*, *J. ensifolius*, *Luzula campestre* varieties, *Brodiaea congesta*, *Calochortus Purdyi*, *Fritillaria lanceolata*, *Erythronium giganteum*, *Camasia Quamash*, *C. Leichlinii*, *Trillium chloropetalum*, *T. ovatum*, *Disporum oreganum*, *Iris tenax*, *Calypso bulbosa*, *Salix lasiandra lancifolia*, *S. Piperi*, *S. Scouleriana*, *Populus trichocarpa*, *Betula Hallii*, *Corylus californica*, *Alnus oregona*, *Quercus Garryana*, *Phoradendron villosum*, \**Rumex acetosella*, \**R. obtusifolius*, \**Polygonum aviculare*, *P. spargulariaeforme*, \**Chenopodium album*, \**Amaranthus retroflexus*, *Montia fontana*, *M. sibirica*, *M. parviflora*, *M. linearis*, \**Cerastium vulgatum*, \**C. viscosum*, \**Stellaria media*, *Arenaria macrophylla*, *Nymphaea polysepala*, *Ranunculus Bongardi*, *R. orthorhynchus*, *Delphinium columbianum*, *Caltha asarifolia*, *Berberis aquifolium*, *Eschscholzia californica*, \**Raphanus sativus*, *Dentaria tenella*, *Cardamine oligosperma*, \**Brassica campestris*, \**Sisymbrium officinale leiocarpum*, *Radicula curvisiliqua*, \**Capsella Bursa-pastoris*, *Ribes sanguineum*, *Philadelphus Gordonianus*, *Saxifraga integrifolia*, *Tellima grandiflora*, *Prunus emarginata erecta*, *Osmaronia cerasiformis*, *Holodiscus discolor*, *Spiraea Douglasii*, *Rubus macropetalus*, *R. parviflorus*, *R. leucodermis*, \**R. laciniatus*, \**Rosa rubiginosa*, *R. nutkana*, *R. pisocarpa*, *Sanguisorba occidentalis*, *Amelanchier florida*, *Crataegus Douglasii*, *Potentilla gracilis*, *Fragaria chiloensis*, \**Cytisus scoparius*, *Lupinus micranthus*, *L. columbianus*, *L. lignipes*, *L. oreganus*, *Trifolium tridentatum*, \**T.*

*repens*, \**T. dubium*, \**Medicago lupulina*, *Vicia americana*, *Lathyrus polyphyllus*, *Lotus micranthus*, *Geranium oreganum*, \**G. dissectum*, \**G. molle*, *Oxalis Suksdorfii*, *Rhus diversiloba*, *Acer macrophyllum*, *A. circinatum*, *Ceanothus sanguineus*, *Sidalcea campestris*, *S. Nelsoniana*, \**Hypericum perforatum*, *Viola glabella*, *V. Howellii*, *Boisduvalia stricta*, *Sanicula Menziesii*, \**Daucus Carota*, *Angelica arguta*, *Oenanthe sarmentosa*, *Ligusticum apiifolium*, *Cornus occidentalis*, *Androsace occidentalis*, *Trientalis latifolia*, *Fraxinus oregana*, \**Convolvulus arvensis*, *Navarretia squarrosa*, *Alloccarya granulata*, *A. Scouleri*, *Mimulus guttatus*, \**Plantago lanceolata*, *Symphoricarpos albus*, *Valerianella congesta*, \**Dipsacus sylvestris*, \**Hypochaeris radicata*, \**Taraxacum officinale*, *Agoseris laciniata*, *Hieracium albiflorum*, \**Crepis capillaris*, \**C. setosa*, \**Lactuca scariola integra*, *Grindelia integrifolia*, *Solidago lepida elongata*, *Eucephalus vialis*, *Aster Hallii*, *Madia glomerata*, *M. racemosa*, *M. sativa*, *Eriophyllum ternatum*, *Achillea Millefolium lanulosa*, \**Anthemis cotula*, \**A. arvensis*, \**Matricaria matricarioides*, \**Chrysanthemum leucanthemum pinnatifidum*, *Senecio vulgaris*, *S. fastigiatus*, *Antennaria Howellii*, *Adenocaulon bicolor*, \**Centaurea cyanea*, \**Cirsium arvense*, \**C. lanceolatum*, \**Arctium minus*.

#### ROGUE-UMPUQUA REGION

In this area are included the main valleys of the Umpqua and Rogue rivers. These streams rise not far from each other near the summit of the Cascade Mountains, and, working westward by very tortuous courses, form a portion of the drainage system of the series of valleys comprising the great trough lying between the Coast Mountains on the one hand and the Cascades and Sierras on the other and extending from Puget Sound to southern California. To the eastward the ascent to the Cascades is gradual, especially from the Rogue River basin, making the boundary of the region here very vague. The Coast Mountains form a fairly well marked limit on the west and the Siskiyou on the south. The Calapooia Divide on the north has been spoken of in connection with the Willamette valley region.

The two valleys are separated by the Rogue-Umpqua Divide, a low mountain ridge running across from the Cascades to the Coast Range, and forming a very narrow intrusion of the Cascade region into the one under consideration. This geographical division of the region into northern and southern sections is associated with certain differences in the floras, but these are not deemed sufficient to justify the ranking of the two as distinct regions.

The Umpqua basin has comparatively little level alluvial land. It consists mostly of bold, rounded hills with narrow valleys along the streams. The soil here is mostly fertile, but there are also many sterile gravelly tracts. The residual soil covering the hills is thin and poor, losing its moisture very rapidly.



The geological formation of this section is complex. The Cascade lavas form the eastern side of the basin, and the western and northern portions are mostly of coarse Eocene sandstones and shales. A little south of the center is a large area of igneous rock (diabase), also Eocene. This is very resistant, often forming steep slopes quite bare of soil. Farther to the south is another area of Cretaceous conglomerates, sandstones, and shales, followed by an area of confused metamorphosed sedimentary formations and igneous intrusives. The character of the vegetation is unquestionably profoundly influenced by the nature of the soil resulting from the disintegration of these various formations.

The valley of the Rogue has a floor of much greater area than that of the Umpqua. Much of this is fertile, but much also is poor and gravelly. The largest level areas are about Medford and Jacksonville. The soil of the higher lands is extremely varied in accordance with the nature of the rock from which it is formed. From the Rogue and its main tributaries the hills rise in places with bold, almost precipitous slopes, in others the ascent is much more gradual. Between the valley floor and the surrounding elevations there is much soil of a colluvial nature, brought down by slides and rainwash. This is varied in character but mostly gravelly or sandy or mingled with rock fragments.

Geologically, most of the Rogue River valley is similar to the southern part of the Umpqua. The eastern and western portions are made up of metamorphosed sedimentary rock, slates, schists, and gneiss of pre-Mesozoic age, and between these is a large area of metamorphosed Jura-Triassic sediments mingled with volcanic flows and intrusives. Over this part of the valley decomposed granitic rock forms a large element in most of the soil.

The yearly precipitation in the Umpqua Valley is about 875 mm., and that in the Rogue, 625 mm. The rainy season is nearly over by the first of June, and summer temperatures are high. These facts, coupled with the loose character of the soil, causing it to yield its moisture promptly to evaporation, give a more decidedly xerophytic aspect to the vegetation than a mere statement of the amount of precipitation would lead us to expect. This condition is of course most marked toward the southern end of the region.

It seems better to consider the two sections of the region separately, in order that we may draw a clearer comparison with respect to their differences as well as their similarities.

The differences between the extreme southern end of the Willamette valley and the northern end of the Umpqua are not striking, but within a few miles of the summit of the divide the vegetation begins to take on a decidedly different facies. The tracts of coniferous woods, mainly *Pseudotsuga taxifolia*, become fewer and retreat from the lower portion of the valley toward the summits of the higher elevations, extending farther down only on the cooler slopes. The lower slopes and summits are mostly occupied by a

somewhat scattered and uneven growth of *Quercus Garryana*, *Quercus californica*, and *Arbutus Menziesii*, nearly all of small size. Mingled with these is a copious undergrowth of shrubs, of which by far the most abundant is *Rhus diversiloba*. Where conditions are slightly less arid, small groups and single trees of *Pinus ponderosa* are frequent, and in places they loosely fringe the *Pseudotsuga* areas.

The lower slopes and gravelly tracts have a copious but short-lived vernal vegetation, which is mostly withered and brown by the middle of June. It consists mainly of annuals or low perennials with bulbs or other fleshy underground parts. There are, however, deep-rooted herbaceous perennials that persist much longer. In well-watered places, such as stream banks, the vegetation more nearly resembles that of the Willamette valley than that of the dry slopes, which is what we naturally expect. To carry further the comparison between the floras of the two sections, it may be stated that the following species included in the list of those most abundant in the Willamette valley are here also at least fairly abundant: *Pseudotsuga taxifolia*, *Aira caryophyllaea*, *Bromus marginatus*, *B. carinatus*, *B. hordeaceus*, *Elymus glaucus*, *Brodiaea congesta*, *Calochortus Purdyi*, *Fritillaria lanceolata*, *Camasia Leichtlinii*, *Trillium chloropetalum*, *T. ovatum*, *Salix Scouleriana*, *Quercus Garryana*, *Phoradendron villosum*, *Rumex crispus*, *Polygonum aviculare*, *Cerastium viscosum*, *Arenaria macrophylla*, *Ranunculus occidentalis*, *Eschscholzia californica*, *Capsella Bursa-pastoris*, *Holodiscus discolor*, *Rosa pisocarpa*, *Sanguisorba occidentalis*, *Crataegus Douglasii*, *Potentilla gracilis*, *Trifolium tridentatum*, *T. dubium*, *Rhus diversiloba*, *Ceanothus sanguineus*, *Boisduvalia stricta*, *Daucus Carota*, *Fraxinus oregona*, *Plantago lanceolata*, *Valerianella congesta*, *Agoseris laciniata*, *Lactuca scariola integra*, *Madia glomerata*, *M. racemosa*, *Achillea Millefolium lanulosa*.

The majority of these are widely distributed, highly adaptable forms, a large percentage of them introduced. Their presence, even in considerable abundance, has little to do with the characteristic aspect of the vegetation.

The following list includes most of those species which occur in the Umpqua Valley and not in the Willamette, or at most only in the extreme southern part: *Gastroidium lendigerum*, *Elymus triticoides*, *Carex bifida*, *C. multicaulis*, *C. gynodynamis*, *Brodiaea Hendersoni*, *Allium Bolanderi*, *Chlorogalum pomeridianum*, *Disporum Hookeri*, *Iris macrostemon*, *Quercus chrysolepis*, *Eriogonum compositum*, *Polygonum californicum*, *Silene campanulata*, *Claytonia bulbifera*, *Arenaria Douglasii*, *Isopyrum stipitatum*, *Arabis Koehleri*, *Thysanocarpus radians*, *Sedum Douglasii*, *Cercocarpus betulaeifolius*, *Prunus subcordata*, *Rosa spithamea*, *Horkelia congesta*, *Trifolium obtusiflorum*, *Lotus humistratus*, *Limnanthes Douglasii*, *L. micranthum*, *Sidalcea Cusickii*, *Sphaeralcea rivularis*, *Vitis californica*, *Lomatium microcarpum*, *Taeniopleurum Howellii*, *Carum erythrorhizon*, *Centaureum Muhlenbergii*, *Microcala quadrangularis*, *Phlox adsurgens*, *Gilia ciliata*, *Navarretia leucocephala*, *N. pubescens*, *Phacelia verna*, *P. Bolanderi*, *P.*

*californica*, *Verbena prostrata*, *Madronella villosa*, *Scutellaria angustifolia*, *Nicotiana Bigelovii*, *Mimulus cardinalis*, *Microseris linearifolia*, *M. procera*, *Eupatorium occidentale*, *Chrysothamnus nauseosus*, *Solidago californica*, *Rudbeckia californica*, *Achyrachaena mollis*, *Calycadenia truncata*, *Cacaliopsis nardosmia*.

Numerous other species of regular occurrence in the Willamette valley are here so much more abundant as appreciably to affect the appearance of the vegetation to one passing over the divide from the northward. Among the most noteworthy of these are *Pinus ponderosa*, *Briza minor*, *Poa scabrella*, *Festuca rubra*, *Elymus Caput-Medusae*, *Sisyrinchium grandiflorum*, *Alnus rhombifolia*, *Quercus californica*, *Eriogonum nudum*, *Lomatium utriculatum*, *Arbutus Menziesii*, *Cryptantha flaccida*, *Trichostima oblongum*, *T. lanceolatum*, *Collinsia Rattani*, *Synthyris rotundifolia*, *Chrysopsis villosa*, *Erigeron decumbens*, and *Wyethia angustifolia*.

The general aspect of the flora of the Rogue River valley is not greatly unlike that of the Umpqua. There is much the same distribution of coniferous forests and open or sparsely wooded slopes. But the former do not consist of a nearly pure growth of *Pseudotsuga*; this, indeed, has retreated mostly to the summits of the highest hills or low mountains, while at lower levels there is a mixture of *Pinus ponderosa*, *P. Lambertiana*, and *Libocedrus decurrens*. The more scantily wooded slopes are occupied largely by *Quercus* and *Arbutus*, but there is a dense undergrowth in most places of *Ceanothus integerrimus*, *Ceanothus cuneatus*, and *Arctostaphylos viscida*. The still lower areas of sterile, gravelly residual and colluvial soil support an abundant though dwarfed vegetation of short-lived annuals and herbaceous perennials. In the most typical localities these appear in rapid succession, mature promptly and disappear, so that the whole aspect of the vegetation over these areas undergoes repeated and striking changes from March to July. The sterile tracts are not clearly distinguishable as a zone from the open forest section, but occur here and there through the latter at its lowest levels. The alluvial soil along the streams, now mostly under cultivation, is naturally covered with a more luxuriant vegetation, quite similar to that of similar situations in the Umpqua valley.

All but a few of those species listed as occurring in the Umpqua valley and scarcely or not at all in the Willamette, are found, and in greater abundance, in the Rogue River valley. A more careful search would probably reduce still further the number of those now known only from the Umpqua section. There are numerous species, however, found in the Rogue valley and not known from the Umpqua. Of these are the following, the list being very far from complete: *Bromus subvelutinus*, *Agropyron spicatum*, *Scirpus lineatus*, *Calochortus uniflorus*, *Lilium pardalinum*, *Fritillaria recurva*, *Erythronium Hendersoni*, *Smilax californica*, *Eriogonum virgatum*, *E. vimineum*, *Amaranthus californicus*, *Arenaria californica*, *Paeonia Brownii*, *Lepidium reticulatum*, *L. oreganum*, *Saxifraga parvifolia*, *Ame-*

*lanchier pallida*, *Horkelia tridentata*, *Lupinus luteolus*, *Trifolium fucatum*, *Astragalus accidens*, *A. pacificus*, *Limnanthes gracilis*, *L. floccosa*, *Rhus trilobata*, *Viola lobata*, *Epilobium oreganum*, *Taraxia graciliflora*, *Leptotaenia californica*, *Eryngium Vaseyi*, *Cornus glabrata*, *Arctostaphylos viscida*, *Frasera nitida*, *Phlox speciosa*, *Navarretia atractyloides*, *Phacelia Rattani*, *Pectocarya pusilla*, *Allocarya cerebriformis*, *Trichostima laxum*, *Madronella reflexa*, *Scutellaria tuberosa*, *Stachys vestita*, *Pentstemon Roezli*, *Collinsia linearis*, *C. bicolor*, *Pedicularis densiflora*, *Orthocarpus lithospermoides*, *Plantago erecta*, *P. tetrantha*, *Galium Bolanderi*, *G. Andrewsii*, *G. Nuttallii*, *Psiloria vergata*, *Crepis occidentalis*, *C. monticola*, *Brickellia californica*, *Aster Chamissonis*, *A. Menziesii*, *A. militarius*, *Hemizonia luzulaefolia*, *Calycadenia multiglandulosa*, *Centromadia Fitchii*, *Helianthus exilis*, *Baeria aristosa*, *Stylocline filaginea*, *Evax caulescens*.

#### SOUTHERN COAST REGION

This comprises the narrow coastal strip from the mouth of the Coquille River southward into California. In area it is the smallest of the several plant regions of Oregon, but its character is well marked.

From the mouth of the Coquille to Port Orford, a comparatively level sandy tract extends from the coast line back for a distance of from two to five miles. Where there is a gradual slope to the beach, the boundary of the region becomes vague, since the maritime influences that give it its character here extend farther inland and die out gradually; elsewhere the definition is sufficiently clear. Topographically the most conspicuous features of this stretch of coast are Cape Blanco and The Heads, near Port Orford. From the botanical standpoint these imposing promontories are extremely interesting. Several small lakes and swamps occur in this section, similar to those of the more northerly coast; there are also some good-sized sphagnum bogs and moderate sand dunes.

Just below Port Orford the character of the coast changes. Beaches disappear, and high bluffs or low mountains rise with steep slopes directly from the sea. This condition prevails for most of the remaining distance to the California line, but there is a considerable beach for several miles above the mouth of the Rogue River, also at the mouth of the Pistol River, and at other points farther south, where dunes and sphagnum bogs are also found.

The more level northern portion of this region, extending as far as Port Orford, consists of a layer of Pleistocene sand capping the Myrtle formation of Cretaceous age, which is made up of conglomerates, sandstones, and shales. The latter, at its more resistant points, *viz.*, Cape Blanco and The Heads, has withstood erosion, developing the picturesque sea cliffs that occur here; elsewhere the underlying formation is not exposed, except at the extreme northern end of the region. Most of the way from Port Orford to the mouth of the Rogue River, the Myrtle formation is not covered by later deposits, but in two or three places it is interrupted by igneous formations.

From the mouth of the Rogue southward, except in a few places where it is covered by later sands, the rock is mainly of metamorphosed sediments of Triassic or Jurassic age.

At the extreme northern end of the region climatic conditions prevail similar to those of the southern portion of the northern coast region, but from Cape Blanco southward the conditions are markedly different. The heaviest winter gales on the Oregon coast occur at Cape Blanco; these are only a little less severe at Port Orford, owing to the conformation of the coast. During summer and early autumn the sky is mostly clear, with strong, cold, northwesterly winds. There is little fog at any season.

From Port Orford southward there is again a marked change. The winds become less severe, the temperatures higher, and clear days fewer. At Brookings, near the southern end of this section, summer temperatures up to 85° F. are common, and fogs often prevail for weeks together. The annual precipitation for the whole region is about 1125 mm., the same as that of the northern coast region.

The vegetation of this region is strongly Californian in character, with an aspect quite different from that of the northern coast. As we pass below the mouth of the Coquille River, numerous species of the beaches and steep seaward slopes come into prominence that occur rarely or not at all above this point, while the zone farthest removed from the sea is dominated by an almost entirely different set of species. One noticeable feature of the flora is the presence of numerous forms that occur inland much farther north, but not on the northern coast.

Among the most characteristic of the beach species are: *Triglochin striata*, *Ammophila arenaria*, *Spergularia leiosperma*, *Sphaerostigma spirale*, *Phacelia argentea*, *Cryptantha leiocarpa*, *Corethrogyne spathulata*, *Tanacetum camphoratum*.

Intergrading with the beach assemblage is that occupying the exposed seaward slopes that are mostly devoid of trees and tall shrubs. With these may be included several species especially plentiful on the steep faces of small rocky islets. Particularly noteworthy in this category are the following: *Juniperus sibirica*, *Brodiaea minor*, *Calochortus maweanus*, *Zigadenus Fremonti*, *Iris Douglasiana*, *Eriogonum latifolium*, *Mesembryanthemum equilaterale*, *Silene multicaulis*, *Arenaria Nuttallii*, *Erysimum capitatum*, *Sedum spathulifolium*, *Dudleya farinosa*, *Heuchera micrantha*, *Lupinus bicolor*, *L. arboreus* var., *Trifolium fucatum*, *T. cyathiferum*, *T. barbigerum*, *Medicago arabica*, *Sidalcea malvaeflora*, *Sanicula arctopoides*, *Torilis nodosa*, *Statice arctice californica*, *Dichondra repens*, *Gilia achilleaefolia*, *Chamissonis*, *Phacelia californica*, *P. malvaeflora*, *Linaria texana*, *Mimulus glutinosus brachypus*, *Castilleja angustifolia* var., *Orthocarpus erianthus*, *Plantago virginica*, *P. subnuda*, *P. coronopifolia*, *Microseris Bigelovii*, *Agoseris hirsuta*, *Grindelia cuneifolia*, *Baeria macrantha*, *Eriophyllum staechadifolium*, *artemisiaefolium*, *Soliva sessilis*, *Helenium Bolanderi*, *Silybum marianum*.

Farther removed from the beach is the usual zone of tall thickets and low woods passing gradually into the forested slopes of the Coast Mountains. Here we miss the dominance of *Pinus contorta*, *Picea sitchensis*, and *Gaultheria Shallon*, and find instead an immense abundance of *Ceanothus thyrsiflorus*, *Garrya elliptica*, *Rhododendron occidentale*, and others. Including the sphagnum bogs in this association, the most characteristic forms are the following: *Carex gynodynamis*, *C. Buxbaumii*, *Scirpus nutans*, *Brodiaea Bridgesii*, *Lilium pardalinum*, *L. Kelleyanum*, *Populus tremuloides* var., *Alnus sinuata*, *Ribes Menziesii*, *Ribes nevadense*, *Rosa Eastwoodae*, *Ulex Europaeus*, *Lathyrus Peckii*, *Ceanothus thyrsiflorus*, *C. laevigatus*, *Angelica tomentosa*, *Garrya elliptica*, *Vaccinium caespitosum*, *Rhododendron occidentale*, *Gentiana Menziesii*, *Polemonium pulcherrimum delicatum*, *Specularia biflora*, *Eriophyllum Harfordii*, *Erechtites arguta*.

#### SOUTHERN COAST MOUNTAIN AND SISKIYOU REGION

A line drawn eastward from the mouth of the Coquille River across the Coast Mountains to the Umpqua valley may be taken as the northern boundary of this region. The southern section of the coastal strip of course forms the western limit. The Rogue-Umpqua region forms most of the eastern boundary, but toward the southern end of the state the Siskiyou Mountains strike across and meet the Cascades.

Much the greater part of this area consists of a confusion of broken mountain ridges with steep and stony slopes and scanty soil. There are a few small valleys, like those along the Illinois River and its tributaries, where a little level land occurs, but even this consists largely of sterile gravelly flats. The maximum elevation is that of Mount Ashland, 2500 meters, on the high north slope of which there is a small amount of perennial snow. Where the Rogue River breaks through the Coast Mountains it forms the most remarkable canyon in the state.

The soil is of course mainly residual, and, the rock from the decomposition of which it is derived being extremely varied, the soil is correspondingly so. The best soils are found in the extreme northern and southern parts of the region and along the western bases of the Coast Mountains.

The geological history of this area is more complex than that of any other portion of the state, and the surface formations are numerous and strongly contrasted. The northern part consists mostly of Eocene shales, sandstones, and conglomerates, overlaid along the western border by marine sands of the Pleistocene. The former narrows toward the south, ending a short distance below Rogue River. To the westward of this narrow strip, which is near the middle of the region from east to west, is a large area of a somewhat similar formation of Cretaceous age, interrupted in various places by altered intrusives (Mesozoic) and pre-Mesozoic metamorphosed sediments—schists and gneisses. Most of the southern half of Curry County

consists of metamorphosed sedimentary rock with volcanic flows and intrusives, and is of Jurassic-Triassic age. The greater part of the Siskiyou section, that is, southern Josephine and Jackson counties, are pre-Mesozoic slates, schists, and gneisses with some basalt, and toward the southeastern extremity of the region in Oregon we find the same formation as occurs in southern Curry County, with small amounts of Cretaceous shales, sandstones, and conglomerates. While in many cases the characteristics, both chemical and physical, of these various formations can be seen to have a profound influence on the character of the vegetation, no discussion of this phase of the subject can be undertaken at this point.

A soil so scanty and so pervious to moisture as prevails over most of this region, coupled with the relatively high summer temperatures and long dry season, produces rather strongly xerophytic conditions. The precipitation ranges from 1000 mm. to 1500 mm., being much higher to the westward, but for the reasons just mentioned this indicates little as to the actual moisture conditions during the growing season. At elevations of 1000 meters and upward, winter snows of considerable depth occur.

The northern part of this region, particularly the west slope of the Coast Mountains, is well forested, mainly with *Pseudotsuga taxifolia* and *Chamaecyparis Lawsoniana*, though the latter has been extensively lumbered off; there is also a mixture of *Abies grandis* and *Tsuga heterophylla*. To the southward the forest becomes more restricted and interrupted as the land rises more abruptly from the coast to the dry sterile summits of the mountains. Toward the southern end of the area the forest again improves as the rise from the sea becomes more gradual. On cooler slopes throughout much of the region, especially at higher altitudes and where soil conditions are a little above the average, well developed forest tracts occur. Though as a whole the forests of this region are much inferior to those of the northern Coast and Cascade regions, it is much richer in coniferous species than any other area of equal size in the state, there being at least seventeen species recorded.

Probably more than half of the total area is covered with a more or less dense growth of shrubs of a great variety of species. Where the ground is extremely stony and the soil most scanty, the growth is very dwarfed; elsewhere it is taller and denser, often nearly impenetrable. Among the most abundant forms are *Quercus chrysolepis vacciniifolia*, *Q. densiflora echinoides*, *Castanopsis chrysophylla minor*, *Umbellularia californica*, *Amelanchier gracilis*, *Ceanothus pumilus*, *Garrya buxifolia*, *Arctostaphylos tomentosa*, and *A. patula*.

The herbaceous vegetation is abundant in species but scant in total quantity. Especially remarkable is the scarcity of grass. Most of the region affords no pasturage whatever for livestock.

The following list includes the most abundant and the most characteristic species of the region; of the former the great majority are more plentiful

than elsewhere in the state, while of the less abundant forms listed, the known range in Oregon is nearly or quite limited to this section.

*Cupressus Macnabiana*, *Sequoia sempervirens*, *Picea Breweriana*, *Pinus attenuata*, *Panicum thermale*, *Carex Brainerdii*, *C. mendocinensis*, *C. luzulina*, *C. scabriuscula*, *Scirpus criniger*, *Allium falcifolium*, *Calochortus Howellii*, *Lilium Bolanderi*, *Erythronium citrinum*, *E. Howellii*, *Hastingsia alba*, *Trillium rivale*, *Narthecium californicum*, *Tofieldia occidentalis*, *Veratrum insolitum*, *Iris bracteata*, *Cypripedium californicum*, *Habenaria aggregata*, *Salix Hindsii*, *Castanopsis chrysophylla minor*, *Quercus Sadleriana*, *Q. Oerstediana*, *Q. chrysolepis*, *Q. chrysolepis vacciniifolia*, *Q. densiflora*, *Q. densiflora echinoides*, *Asarum marmoratum*, *Umbellularia californica*, *Eriogonum pendulum*, *E. siskiyouense*, *E. ursinum*, *Lewisia Howellii*, *Silene longistylis*, *Arenaria Howellii*, *Delphinium nudicaule*, *Berberis pumila*, *Vancouveria chrysantha*, *V. parviflora*, *Dicentra glauca*, *Dentaria gemmata*, *D. pauciflora*, *Arabis purpurascens*, *Draba Howellii*, *Streptanthus glandulosus*, *S. orbiculatus*, *Darlingtonia californica*, *Gormaniana laxa*, *Saxifraga fragarioides*, *Ribes Marshallii*, *R. montanum*, *Parnassia californica*, *Tellima campanulata*, *Amelanchier gracilis*, *Horkelia sericata*, *Thermopsis argentea*, *Lupinus mucronulatus*, *Astragalus catalinensis*, *Trifolium Breweri*, *Vicia californica*, *Polygala californica*, *Rhamnus occidentalis*, *Ceanothus pumilus*, *Viola cuneata*, *V. occidentalis*, *V. lobata*, *Epilobium rigidum*, *Zauschneria latifolia*, *Sanicula Peckiana*, *Lomatium Nelsonianum*, *L. Howellii*, *Euryptera Howellii*, *Garrya buxifolia*, *Sarcodes sanguinea*, *Vaccinium coccineum*, *Arctostaphylos tomentosa* varieties, *Arctostaphylos patula*, *Gentiana bisetacea*, *Convolvulus polymorphus*, *Gilia palmipars*, *Phacelia dasphylla ophitidis*, *Eriodictyon glutinosum*, *Cynoglossum occidentale*, *Lithospermum californicum*, *Madronella purpurea*, *M. ovata*, *Antirrhinum Breweri*, *Pentstemon azureus*, *Collinsia Torreyi*, *Castilleja elata*, *C. brevilobata*, *Galium californicum*, *Hieracium Bolanderi*, *Brickellia Greenei*, *Aplopappus congestus*, *Erigeron confinus*, *E. nudatus*, *E. Aliceae*, *Aster tomentellus*, *Eriophyllum lanceolatum*, *Helenium Bigelovii*, *Senecio hesperis*, *S. trigonophyllus*, *Antennaria suffrutescens*, *Cirsium acanthodontum*.



## SOME CASES OF APPARENT SINGLE FERTILIZATION IN BARLEY

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In the angiosperms, double fertilization, so far as known, usually occurs. As is well known, the megaspore develops into the female gametophytic generation. In the final stage there are, among other structures, one female gamete and two polar nuclei which are directly concerned in the acts of fertilization. The microspore divides, producing a vegetative and a generative cell, the latter sooner or later dividing to form two male gametes. As the pollen tube grows toward the embryo sac, the male gametes move along with the forward growth of its tip and are set free within the embryo sac when the pollen tube has penetrated the latter. The egg nucleus unites with one of the male gamete nuclei, and the zygote containing the fusion nucleus, by successive divisions, produces the embryo. The remaining male gamete nucleus unites with the two polar nuclei of the embryo sac; the primary endosperm cell, now containing this nucleus formed by a triple fusion, develops into the endosperm.

The condition known as *xenia* exists when the endosperm shows, by one or more characters, the effect of the union of the two female polar nuclei with a male gamete from a parent possessing the character or characters in question.

In barley we have some strains with colorless aleurone layers and others with colored aleurone layers, and, as the aleurone layer is a product of the endosperm, we should expect *xenia* here also. In hybrids made in 1916 the senior author found absolute evidence of this phenomenon. A cross was made between the two-rowed variety Svanhals and the six-rowed variety Manchuria, the former being used as the female parent. The aleurone layer of Svanhals is colorless, while in the strain of Manchuria used in the cross the aleurone is blue. The kernels of the Svanhals parent pollinated with Manchuria pollen were as blue as those of the Manchuria itself. In the same year were harvested at Moccasin, Montana, some later generations of certain crosses. In the plants from the heterozygous seeds of this hybrid material were found heads containing both blue and white kernels. The occurrence of blue and white seeds in the same spike had been noted also by N. C. Donaldson of the Office of Cereal Investigations, then in charge of the field work at Moccasin.

In taking the data for the studies pursued on kernel-development in barley it has been necessary to examine rather minutely many thousands of kernels, some of which showed certain interesting abnormalities. Two

striking sorts have appeared which seem to be connected with the question of double fertilization. Six kernels have been found which seem to lack the embryo, and a much greater number have been observed which were deficient in endosperm content. Kernels of both types were sectioned and studied.

The kernels lacking the embryo appear normal except for the presence of a slight depression in the place usually occupied by the scutellum. One of those labeled "without embryo" did possess that structure, although it was small and abnormal. In the other five kernels there was found no trace at all of any mass of cells which could be considered embryo tissue.

Longitudinal vertical sections of the embryo-lacking kernels (Pl. VI, fig. 1) were made and compared with corresponding sections of a normal barley kernel (fig. 2). In the former we find a normal endosperm, consisting of an interior of starch cells enveloped by a normal aleurone. The aleurone extends toward the proximal end of the kernel on the dorsal and ventral surfaces to the same distances as in the normal kernel, and the seed coats are complete and unbroken (fig. 3), as would be expected, since they are of maternal origin. The only differences evident lie in the region normally occupied by the embryo.

In the embryoless kernel there is a small cavity just beneath the seed coats where the embryo normally would be located. This cavity contains some disorganized tissue consisting of cells from probably more than one source. The loose layer just within the seed coats at the proximal end (figs. 1, 3) may contain remnants of the wall of the nucellus and the single row of aleurone cells which encircles the embryo in the normal kernel. There also are cells of irregular outline appearing singly or in clumps in all the specimens. These are not well localized and show signs of senescence, although one pair of cells has been found with a thin cell wall apparently newly laid down between them (fig. 4, *a*). These cells may very well be the progeny of the antipodals present in the embryo sac at the time of fertilization, or they may be the remnants of an aborted embryo.

The second or endosperm-deficient type, which, from its appearance, was called the "watery kernel," has a very characteristic appearance. It is recognizable at a much earlier age than is the embryo-deficient type, as the scutellum of the normal barley grain is not apparent in unsectioned kernels until the twelfth day after pollination and then only dimly so. Consequently, the disparity in numbers found is no evidence that the two types do not occur with equal frequency.

The endosperm-deficient or watery kernel is slightly lighter green than the normal and is swollen and yielding to the touch. Upon rupture of the wall, a watery liquid distinctly sweet to the taste flows out and the body collapses. Several of these watery kernels were sectioned. The seed coats are normal, but there is neither aleurone layer nor starchy endosperm. In two such kernels normal embryos were found (fig. 5). In the other two

there was some difficulty experienced in cutting, and, while no embryo was found, there was evidence that it had been present but had dropped out in the process of sectioning. The tissues where the endosperm should be are scanty and scattered unevenly through the space. The cells are nucleated and may very well be of nucellar origin. Such tissues normally would have been pushed to one side and greatly compressed by the growing endosperm, but, owing to the plenteous space and food material available, it might have been possible for them to continue to develop further than would have been possible normally. In these cells there are found early stages in starch-grain-formation (fig. 6).

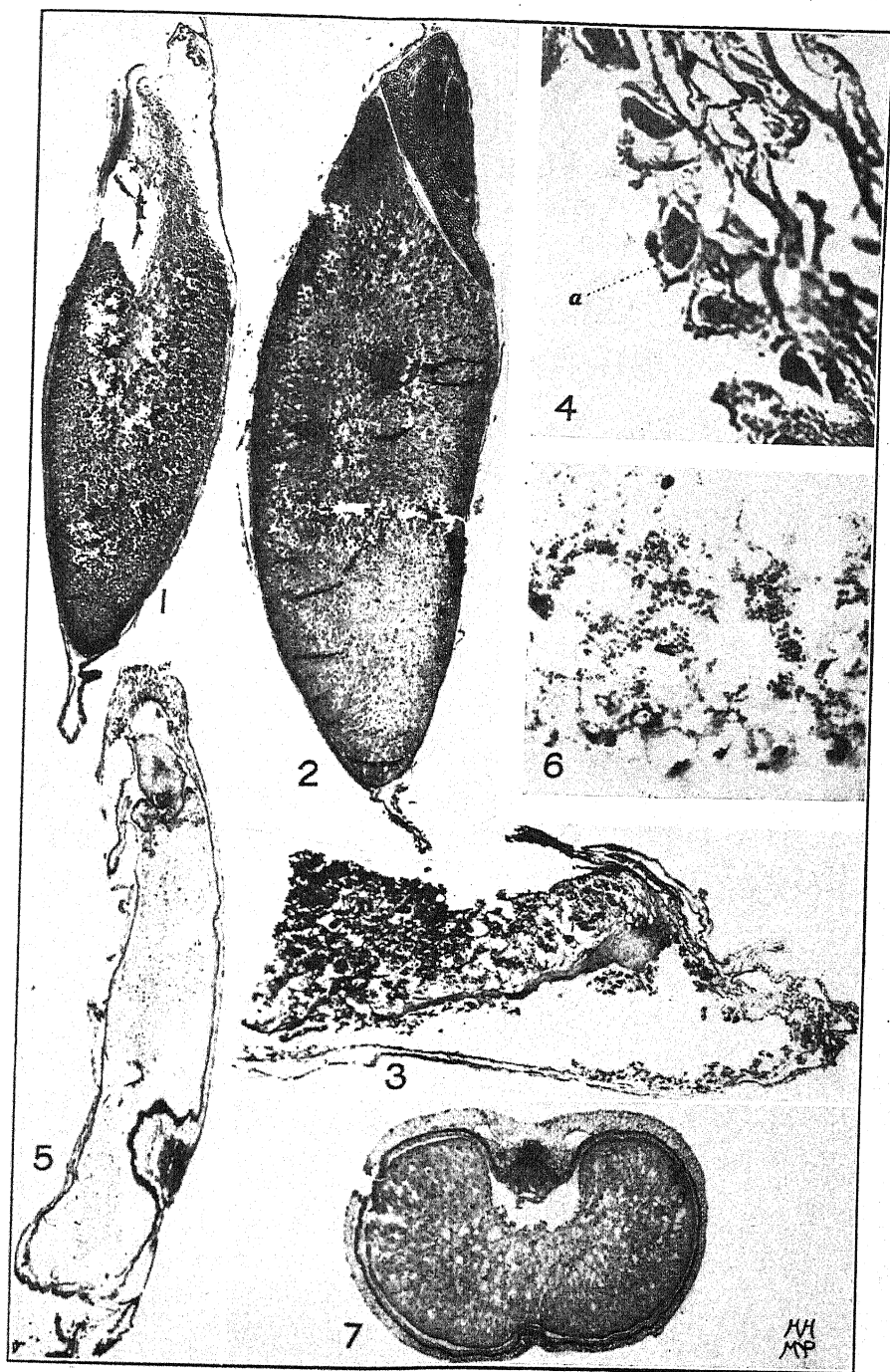
The cross section of a normal barley kernel of about the same age as the endosperm-deficient kernel shown in figures 5 and 6 is shown for comparison in figure 7. Here the aleurone is a distinct and well developed layer which does not appear in the abnormal kernel, and the endosperm is densely packed with nucleated cells containing starch grains of various sizes. In the "watery" kernels the tissue within the cavity normally occupied by the endosperm is disorganized and composed of scattered cells which contain young starch grains.

One hypothesis to explain these two phenomena is obvious, namely, that but a single fertilization occurred where either abnormality resulted. In the case of the embryo-deficient kernel it is probable that the egg nucleus was not fertilized, while the endosperm developed in a practically normal manner without a simultaneous development of embryo. In the case of the "watery" kernel the embryo developed normally while there was probably a failure in the fertilization producing the endosperm.

Of course, there may have been initial fertilization with some later circumstance inhibiting the development of the missing part or disrupting it entirely. However, this seems rather unlikely, because, as may be seen in the section of the embryoless kernel, there appears to be no tissue of embryonic origin present, and the apparently normal development of the embryo in one, and of the endosperm in the other, tends to minimize the likelihood of any injury in the embryo sac which would destroy one body and permit an adjacent one to develop normally.

In the case of the endosperm-deficient kernels, the very scanty quantity of tissue within the nucellar wall, by no means filling the space available, would lead one to believe that there had been no endosperm-development whatever. These cells all have the power of division, and any injury to the growing endosperm would necessarily include most of the cells to prevent some sort of tissue being found.

It was at first thought possible that the enzymes necessary for starch-formation were not present in the endosperm-deficient kernels, with the result that the carbohydrates continued to exist as the sugars which were transported to the seed from their source of manufacture. The presence of young starch grains in the tissue extraneous to the embryo disproves this hypothesis.



HARLAN AND POPE: SINGLE FERTILIZATION



A cytological study of the beginnings of these phenomena would be desirable, but, as neither abnormality occurs with sufficient frequency to seem to justify the great amount of labor which would be necessary, such work has not been done.

OFFICE OF CEREAL INVESTIGATIONS,  
BUREAU OF PLANT INDUSTRY,  
U. S. DEPARTMENT OF AGRICULTURE,  
WASHINGTON, D. C.

#### DESCRIPTION OF PLATE VI

FIG. 1. Longitudinal vertical section of an embryo-deficient kernel showing normal endosperm and aleurone.  $\times 11$ .

FIG. 2. Longitudinal vertical section of a normal kernel aged 23 days.  $\times 11$ .

FIG. 3. Longitudinal vertical section of the proximal end of an embryo-deficient kernel, showing unbroken seed coats and scattered cells underneath.  $\times 35$ .

FIG. 4. Portion of figure 3 under greater magnification, showing recent cell division in the embryo cavity.  $\times 285$ .

FIG. 5. Longitudinal vertical section of an endosperm-deficient kernel showing normal embryo, disorganized and scattered tissue, and collapsed walls. Aged approximately 12 days.  $\times 11$ .

FIG. 6. Section of tissue with endosperm cavity from the same kernel from which figure 5 was drawn, showing early stages of starch-formation.  $\times 147$ .

FIG. 7. Cross section of normal kernel aged 12 days.  $\times 18$ .

Figures 1, 2, and 5 were photographed with a 72-mm. micro-tessar lens; figures 3 and 7 with a 32-mm. micro-tessar lens; figure 4 with a Leitz 4-mm. apochromatic lens; and figure 6 with a Leitz ocular no. 1, objective no. 4.

The writers are indebted to E. G. Arzberger and E. F. Artschwager of the Bureau of Plant Industry for use of apparatus and assistance in making the photographs.

## FRANK SHIPLEY COLLINS

1848-1920

W. A. SETCHELL<sup>1</sup>

Frank Shipley Collins was born in Boston, Massachusetts, February 6, 1848, and died in New Haven, Connecticut, on May 25, 1920. He was graduated from high school in 1863 and entered on a business career in 1864, which he continued with slight interruption until retired and pensioned in 1913. Through war-time call from his old firm he resumed connection with business along the lines of his previous experience in 1918, was variously situated with them, and died in harness. In 1875 he married Anna Lendrum Holmes of Little Falls, New York, who with two sons survives him. His botanical and other scientific work occupied only his "leisure" hours. His botanical interests were wide, but he early directed his attention to the algae, particularly to the marine species at first, but later to those of the fresh water and to aerial forms as well. He began to publish notes on algae in 1880, when in his thirty-third year. From that time on he was an active factor in the advance of American phycology.

Frank Shipley Collins was the son of Joshua Cobb Collins and Elizabeth Ann (Carter) Collins. His paternal grandparents were Michael Collins and Dorcas D. (Cobb) Collins of Eastham, Massachusetts, on the Cape Cod Peninsula, where both the Collins and the Cobb families had been long established. The old Collins house, built over two centuries ago, when hand-worked lumber only was available, is still standing and is occupied by the widow of Frank Shipley Collins with the title vested in his grandson. It is a quaint old mansion still retaining much of its early colonial character. Michael Collins held many offices of trust in Barnstable County, Massachusetts. The Carters and their relatives were Charlestown people and numbered "yeoman" manufacturers, merchants, and sea captains among their ilk. The pedigrees on all sides seem to have run back to Massachusetts colonials of the seventeenth century.

Frank Collins, being a delicate child without desire for outdoor play, spent much of his time in his earlier years over books and listening to instructive stories told by his mother or her two sisters, Miss Sarah Putnam Carter and Mrs. Abigail (Carter) Shipley. Both his aunts taught in the Charlestown Female Seminary, where his mother had also been a teacher. Their subjects included English, Greek, Latin, French, mathematics, and botany, and these became familiar to the subject of our sketch early in life. It was through this grounding that he was able to carry on so successfully

<sup>1</sup> In behalf of a committee of the Botanical Society of America, consisting of M. A. Howe, W. A. Setchell, and I. F. Lewis.



Very truly  
Yours  
J. Frank A. Ballman





his scientific work in spite of the lack of a university training. He went to a very exclusive private school in Malden at the age of three, and later, the family having removed to Malden after the death of his father, he attended the public schools of the same city, being graduated from all classes with honors and at the same time continuing his home studies in Greek, Latin, French, mathematics, and astronomy. He was graduated from high school in 1863 in his sixteenth year, but suffered so much from asthma for about two years that he could not indulge in active occupation of any kind, being unable to lie down for much of the time. During these years he was enabled to study harmony and the pianoforte, and through contact with an able teacher of music, a talented man of charming personality and one who had traveled much, his attention was directed to opera, concerts, recitals, etc., of the best sort. His mother and aunts desired to have him enter Harvard University and the family means were ample for that purpose, but his grandfather Carter, a practical man, decided that work was better, and he entered upon a business career. After various experiences, mostly disappointing, and the family means having been greatly depleted, he finally, having qualified as an expert bookkeeper, entered the employ of the Malden Rubber Shoe Company, as ticket clerk, with the object of learning the whole system of laying out the separate pieces of boots and shoes to be made the next day by the individual makers. It was a delight to him to study out new forms and eliminate clumsy and unnecessarily long "tickets." He remained in this office, constantly improving methods, as manager, for over thirty years, and after retirement was recalled by the exigencies of war to take up the work anew as a sort of efficiency expert.

The formal education of Frank Shipley Collins was not continued beyond the high school, as has been indicated above, but he was naturally studious and seems to have continued the self-education begun at home under his mother and aunts far beyond the ordinary limits. Even in later years he added Spanish to his linguistic acquirements of Latin, French, and German, because he desired to become acquainted with the Spanish novels of the day. Although neither an artist nor an expert musician, he took the keenest interest in the art exhibits and the work of artist friends as well as in public and private musical performances, having been a constant attendant of the concerts of the Boston Symphony Orchestra as well as the chamber concerts, recitals, etc., for which Boston and its vicinity afforded such a wealth of opportunity. His mental equipment was of the alert and progressive order and led him along many paths. He was at one time much interested in theosophy and, besides acquiring a considerable library on the subject, was very active in discussions and correspondence on the subject. It was this mental alertness, combined with indomitable energy and perseverance, that led Collins to devote his spare time to what might be called recreational work along scientific lines. We find him early (at least some years before 1880) associated with George Edward Davenport and Lorin Low Dame of

Medford in the work and particularly the excursions of the Middlesex Scientific Field Club, which later developed into the Middlesex Institute, under whose auspices Dame and Collins published (1888) their "Flora of Middlesex County." Collins had evidently imbibed the elements of botany from his aunt, Mrs. Simon G. Shipley, but music and art absorbed at first his interests outside of business. After his marriage he was led to take up marine algae through his wife's acquaintance with a collector and merchant of "sea mosses." Mrs. Collins, who had come into contact with Mrs. Maria H. Bray at Magnolia, Massachusetts, had assisted Mrs. Bray in gathering seaweeds and preparing them for sale to her summer boarders. Mrs. Bray and her husband had previously been keepers of the lighthouse on Thatcher Island. One day Mrs. Bray read, in one of the occasional newspapers which had come her way to lighten the tedium of the isolated life at Thatcher Island, an advertisement by someone in the central western portion of the United States, offering most attractive exchanges in return for sea mosses. She answered the advertisement and received directions for collecting and mounting. The collecting and mounting provided her with a wonderful new interest. At Ptilota Cafe, whither she removed later, Mrs. Bray sold the mounted "sea moss" cards, each of which was labeled with the botanical name. Mrs. Collins called Frank Collins' attention to these cards and he became interested. He soon realized that the naming of these cards was very defective, and with his characteristic efficiency started to investigate, soon becoming immersed in what was destined to be his specialty.

We must remember that at the time Collins began to collect and study algae but little was known of our American species, either marine or fresh-water. Harvey had given us the magnificent but woefully incomplete "Nereis Boreali-Americana" (1853-1858). On marine species, D. C. Eaton and F. W. Hall, his pupil, had published some lists, as had Farlow, but there was nothing adequate in the way of local literature. Farlow's "New England Algae" did not appear until 1881. As to fresh-water forms, the more general literature applied also locally, but the works of Wood and Wolle were neither complete nor trustworthy. After 1881, however, with the "Marine Algae of New England" to guide and stimulate, Collins' reaction was definite and positive. He then began the issue of the series of "Notes," which are models of their kind. As early as 1879 he delivered an address before some club on the subject of marine algae, and then, to please his aunt, Mrs. Shipley, who could not attend the meeting, he copied out his discourse, illustrated it with specimens of algae, and presented it to her in the form of a handsomely bound copy. We find that his botanical education must have advanced sufficiently thoroughly and considerably, so that by 1882 he began the series of publications which indicated his passing from the tyro class and ranged him with the authoritative workers, adding particularly to our regional knowledge with accuracy and precision.

Collins was a collector of the very best type. No trip was too long or too venturesome, no locality too remote or too disagreeable; in winter he pierced the ice, and in summer he braved the most trying heat. He followed the phenologic activity of his plants in particular localities, in many of them season by season and year after year, obtaining at first hand knowledge and experience of the stages and life histories of the species he was interested in. His exploration of the New England coast began early; just how early is not apparent, but his collection book begins with the enumeration of algae collected on a trip to Lynn Beach in October, 1878. The entries from that time on indicate how much he was able to get about, and from Falmouth, Massachusetts, to Mt. Desert, Maine, he explored the New England coast, making complete collections whenever possible and preparing his specimens with great care. Later he extended his territory to Newport, Rhode Island, and later still, after the beginning of his acquaintance with another enthusiast in his line, Isaac Holden of Bridgeport, Connecticut, an amateur phycologist of his own aggressive type, he made himself personally familiar with the algae of various localities in the region of Long Island Sound. His knowledge of the marine flora of New England was unrivaled, and in his later years he spent his summer vacations at the Tufts Marine Biological Laboratory at South Harpswell on the Maine coast or at the Marine Biological Laboratory at Woods Hole, Massachusetts. No one had a better knowledge of the marine flora of the coast of New England than Collins possessed, and he had himself observed living and personally collected nearly every species recorded. His additions alone increased our regional knowledge of the marine algae of this coast over 100 percent.

Not only was he an untiring and extensive collector, but he was able to utilize other collectors, both amateur and professional botanists, on the New England coast and on other coasts as well. As early as the nineties, he received collections of rough-dried algae of the Pacific Coast from Miss M. M. Miles, from Mrs. A. E. Bush, from Mrs. J. M. Weeks, from G. W. Lichtenthaler, and later from Mrs. M. S. Snyder and others. These were soaked out, mounted, studied, and exchanged or issued in various "Algae Exsiccatae." Dr. Christ of Basel and Miss Julia Houegger sent very considerable collections from the Canary Islands; Mrs. Cora E. Pease and Miss Eloise Butler collected for him in Jamaica, as did also Dr. J. E. Duerden; Miss C. Messina and Mrs. G. A. Hall at Key West and on the Florida Coast, Miss Eloise Butler and Miss Polley in Minnesota and on Vancouver Island, as well as others. In soaking out and working over these collections, Collins obtained experience of a very considerable portion of the coast of the United States in a much more effective way than could possibly have been gained from individual herbarium specimens, and he used the duplicates in exchange to enrich his constantly growing herbarium.

Frank Collins was fond not only of local botanical excursions, but also of longer trips. "At twenty-five and out of a job," writes Mrs. Collins, "he

borrowed a thousand dollars of his reluctant and scandalized grandfather Carter and took a seven months' trip to Europe." His interests in botany at this time (1873) were not sufficient to be a feature in inducing his visit, but he devoted himself to music and art. He made several trips to Bermuda, collecting not only algae but other plants, particularly flowering plants for different herbaria, as well. He put into order in his later years the algae of the Farlow herbarium, those of the Boston Society of Natural History, and those of the Missouri Botanical Garden. His own algal herbarium was, after his death, purchased by Dr. N. L. Britton and presented to the New York Botanical Garden.

Collins' work was largely regional, at first confined to New England but later extending along the Atlantic coast to Bermuda and to Jamaica, westward to Vancouver Island, and northward to the American Arctic. At the time of his death he was at work on two projects, an account of the marine algae of the Philippines and a manual of the marine algae of New England. His earlier publications were in the form of notes on additions to the marine flora of New England and critical determinations of its species. In 1888 he issued, in connection with L. L. Dame, "A Flora of Middlesex County," and in the same year he contributed an account of the marine algae to Maria L. Owen's "Catalogue of Plants of the County of Nantucket." In 1894 he prepared the account of the marine algae for Rand and Redfield's "Flora of Mount Desert;" in 1901, his "Algae of Jamaica;" in 1913, his "Marine Algae of Vancouver Island;" and in 1917, in connection with Rev. A. B. Hervey, his "Algae of Bermuda." These and other projects led him to critical work on various genera and families, particularly of the Chlorophyceae, culminating in his most considerable publication, "The Green Algae of North America," and its supplements. He prepared at different times accounts of various families of North American algae, a few of which are in definite manuscript form, but found the task of such extended work impossible of completion because of the incomplete state of our knowledge of certain coasts.

His collecting, exchanging, and general handling of specimens led him to take a great interest in published sets of algae, or algae exsiccatae. He contributed to the classic distribution of Wittrock and Nordstedt and to Hauck and Richter's "Phykotheke Universalis," and finally undertook, in connection with Isaac Holden and W. A. Setchell, the onerous task of issuing the "Phycotheca Boreali-Americana." The successful issue of the numerous fascicles, including the handling of over 200,000 specimens, is due to the energy and perseverance of Collins, his co-workers assisting, but the detail of assembling, sorting, preparing labels, title pages, covers, and make-up as well as attending to the necessary financial details, all fell upon him. It was his ambition to have issued fifty of the ordinary fascicles and at least six of the larger size. Through his care, this published set is more fully representative of North American species than it otherwise could possibly

have been. He was most successful in enlisting students of algae in various portions of the country towards making the distribution more complete.

Although Collins, as has already been mentioned, was compelled to occupy himself during the greatest portion of his lifetime with business pursuits quite apart from his hobby of marine algae, yet his accomplishments in the lines of botany and particularly in the realm of phycology brought him the respect and esteem of the highest authorities in his line. His correspondence with Dr. Ed. Bornet of Paris began in 1888 and continued until Bornet's death. His correspondence with Hauck, Richter, Nordstedt, Traill, Holmes, Weber-van Bosse, Gomont, Sauvageau, and other European workers helped him to render his work more accurate and effective as well as to assist in the preparation of many important monographs. His relation to Farlow, especially, and to other American botanists was of the friendliest and of mutual helpfulness. He was a member of the Middlesex Institute, of the Boston Society of Natural History, of the Massachusetts Horticultural Society, of the New England Botanical Society (president for three years), corresponding member of the Torrey Botanical Club, and fellow of the American Academy of Arts and Sciences. He received the degree of M. A. (*honoris causa*) from Tufts College in 1910, and was appointed an associate in the University Museum of Harvard University. Altogether he was of the rare type of conscientious business man who achieves in his amateur connection with science those accomplishments of which a professional may well be proud. A thorough gentleman, kindly and generous in disposition, a loyal friend, a most helpful co-worker, ardent in the pursuit of knowledge of the most satisfying type, Frank Shipley Collins has presented to the scientific world an example of what the amateur may accomplish and has left behind him a host of sorrowing friends and comrades in the work who feel his loss most deeply and who testify to his sterling worth and accomplishments. His memory is perpetuated among the algae by several namesakes, of which *Phaeosaccion Collinsii* Farlow, a most interesting species of the brown algae, and *Collinstella tuberculata* Setchell and Gardner of the green algae may especially be mentioned.

I am deeply indebted to Mrs. Frank S. Collins for information on many important matters included in the foregoing account.

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## A TETRAMEROUS SPECIES, SECTION, AND SUBGENUS OF CAREX

HAROLD ST. JOHN AND CHARLES S. PARKER

(Received for publication October 27, 1924<sup>1</sup>)

The genus *Carex*, with its enormous total of from 800 to over 1000 species<sup>2</sup> and its great variety of form, has long attracted the attention of specialists and the interest of students. Although this diversity shows itself in the number, arrangement, and sexuality of the spikes and in the size and appearance of perigynium and achene, yet one set of characters has run constantly throughout the genus. All known species of *Carex* have either two stigmas, in which case they develop a flattened lens-shaped achene, or three stigmas, in which case the achene is trigonous.

On the wooded slopes of Cedar Mountain (or Moscow Mountain), Latah County, Idaho, a low stoloniferous species of *Carex* is abundant. In the spring of 1922, when determining their botanical specimens, Virgil Argo and Charles S. Parker, both students at the State College of Washington, noticed that this *Carex* had four stigmas. Of course this raised the question, would the plant develop a four-sided achene or would it fail to mature fruit? A single immature specimen from the State of Washington was known to Professor C. V. Piper at the time of the publication of his *Flora of Washington*, and included as the sole record for *Carex Richardsonii* R. Br. Eight years later this determination was changed to *Carex concinnoides* Mackenzie.<sup>3</sup>

Since this beginning the authors and several of the botanical students at the State College of Washington have made further collections, adding eight new localities for Washington. In addition, field observations have shown that this sedge is common in the woods of northwestern Idaho and in eastern Washington wherever there are yellow pine and Douglas fir woods, from the Blue Mountains up to the Pend Oreille River near the Canadian boundary, then west through Ferry and Okanogan Counties to the Cascade Mountains, and south at least as far as Ellensburg.

In the field the senior author has examined growing plants by the hundred and found only one plant showing other than four stigmas. He has collected large sets of the plant in flower and in fruit. These show the achene to be olive brown, 2.8 mm. long, with a slender stipitate base, the body a slightly flattened ellipsoid having four distinct ridges or angles running

<sup>1</sup> Published, at the expense of the authors, out of the order determined by the date of receipt of the manuscript.

<sup>2</sup> G. Kükenthal in *Pflanzenreich*, IV, fam. 20, p. 68 (1909) makes the former estimate; while K. K. Mackenzie in *Erythea* 8: 7 (1922) makes the latter.

<sup>3</sup> Piper, C. V., and Beattie, R. K. *Flora of southeastern Washington and adjacent Idaho*, p. 47. 1914.

from tip to base. The plant clearly belongs in the tribe Cariceae of Benthams and Hooker, or the Caricoideae of Kükenthal. Here it comes closest to *Uncinia* and to *Carex*. Both have an urn-shaped, nearly closed perigynium around the pistillate flower. *Uncinia* is distinguished by having an exserted hooked rhachilla. Then this sedge which lacks the exserted hooked rhachilla resembles most closely a *Carex*. Except for the symmetry of its pistillate flowers it would fall under the subgenus *Eucarex* and the section *Digitatae* of that genus. It would be easy and plausible to describe the plant as a new genus, but the writers feel that would obscure rather than clarify the true relationship. If *Carex* can have members with three, as well as those with two stigmas, why should it not be extended to receive a species with four, which is in every other way a *Carex*? Although tetramerous flowers are usually those of dicotyledons, yet there are a few well recognized monocotyledons with such flowers. For instance, there are the *Najadaceae* and *Potamogetonaceae*, while in the *Cyperaceae* they occur in *Elynanthus*, *Gahnia*, and *Lagenocarpus*. To make possible the recognition of this plant as a *Carex*, it is proposed that the generic description of *Carex* be changed to read: *stigmas*, 2, 3, or 4; *achene lenticular, trigonous, or tetragonous*.

To the great surprise of the authors, this *Carex* is not a new species. It is *Carex concinnoides* Mackenzie.<sup>4</sup> The type specimen was from Columbia Falls, Montana, and cotypes were listed from Moscow Mountain, Idaho; the Selkirk Mountains and Deer Park, British Columbia; and Banff, Alberta. The original description does not mention the number of stigmas, or the shape of the achene. It is inferred that the author thought it had three stigmas and a trigonous achene, for he discussed its closest relatives, *C. concinna* and *C. Richardsonii*, members of the section *Digitatae*, which contains only species with three stigmas and trigonous achenes. Since this original publication, various authors have treated the plant. In 1914 Piper and Beattie<sup>5</sup> include this species in their key as having three stigmas and a 3-angled achene; in 1915 Professor J. K. Henry also keys it on this basis;<sup>6</sup> in 1917 K. K. Mackenzie definitely includes it in the *Digitatae*, which he defines as having this character;<sup>7</sup> in 1922 Mackenzie does likewise in his "Monograph of the California Species of the Genus *Carex*,"<sup>8</sup> and with this is a clear illustration (fig. 27) prepared under the direction of Dr. W. L. Jepson, showing three stigmas; in 1923 Mackenzie keys it and describes it as having three stigmas and triangular achenes, but the accompanying illustration does not show either.<sup>9</sup> This review of the interpre-

<sup>4</sup> Bull. Torrey Bot. Club 33: 440-441. 1906.

<sup>5</sup> Piper and Beattie, *loc. cit.*, p. 44.

<sup>6</sup> Henry, J. K. Flora of southern British Columbia, p. 56. 1915.

<sup>7</sup> See Rydberg, P. A. Flora of the Rocky Mountains, pp. 131, 132. 1917.

<sup>8</sup> Mackenzie, K. K. *Erythea* 8: 56. 1922.

<sup>9</sup> See Abrams, L. Illustrated flora of the Pacific states 1: 282, 321. 1923. In a letter from K. K. Mackenzie, dated April 21, 1924, he states: "For your assistance I might say that my recollection is that the illustration of *Carex concinnoides* in Abrams' Illustrated

tation of the characters of *Carex concinnoides* by the several authors shows that their verdict is unanimous, that the species has three stigmas and trigonous achenes. Against such testimony by so many and such eminent botanists, the authors hesitate to announce a contrary opinion. Yet such is the result of their study, and they have the temerity to make it known.

As already stated, extensive field study has shown *Carex concinnoides* as it grows in Washington and Idaho to be constant in having four stigmas and a tetragonous achene. Mr. St. John has borrowed the material of this species in the private herbarium of W. N. Suksdorf. It consisted of one sheet from Mica Peak, Washington. The specimens were young, showing clearly the four stigmas. Mr. Suksdorf has the plant from this same collection in cultivation at his home at Bingen, Washington, where it grows well, flowers, but has never fruited. Mr. St. John has borrowed the material of this from the Gray Herbarium, for which he is very grateful to Dr. B. L. Robinson. This totals nine sheets, eight of which have been verified by Mr. Mackenzie as *C. concinnoides*. All but one, including two cotypes, showed either four stigmas or a tetragonous achene. This one is *H. N. Bolander 6478*, Red Mountains, California, the only collection known from that state. There is a duplicate of this in Washington, D. C.; but both are so young that no stigmas are showing. However, it seems to be perfectly typical material. The first specimen cited from the state of Washington by Prof. Piper and verified by Mr. Mackenzie, *R. M. Horner 209*, Blue Mountains, April 24, 1897, clearly shows 4 stigmas.

Mr. Parker has studied the collection at the U. S. National Herbarium, where there are three sheets, which have been verified as *Carex concinnoides* by Mr. Mackenzie. Two of these showed four stigmas, the other one being very immature. In the same herbarium, classified as *Carex Richardsonii* R. Br., he found five sheets which are distinctly *Carex concinnoides*. From the same locality as the type sheet, but composed of more mature plants, was one collected by *R. S. Williams 953*, June 7, 1893. This sheet carries three plants, two of which show 4 stigmas and one has well matured tetramerous achenes. The other four sheets were from the border of Emerald Lake, altitude 4300 feet, *J. Macoun*, July 1, 1904; material young but showing 4 stigmas: mountain top, valley of Fraser River, *J. Macoun*, May 21, 1875; this sheet contained three very immature plants but two displayed 4 stigmas: near Banff, lat. 51° 11', long. 115° 34', *J. Macoun*, June 26, 1891, a sheet of three plants, two being very immature, one showing 4 stigmas: Blue Mountains, *Robert M. Horner 648*, April 4, 1897, a sheet of very young material but showing 4 stigmas.

Mr. Parker also examined the material in the New York Botanical Garden, where the type specimen is preserved. On the type sheet there are three plants. Two show achenes quite well developed but not fully matured. Flora is taken from the type specimen." Mr. Parker found a notation to this effect on the type sheet.

The third plant, which is younger, distinctly displays four stigmas. The achenes inclosed in the pocket on the sheet, which were taken from the most mature plant, are tetramerous. This character is not as distinct as in the more mature specimen of *H. St. John* 3249, June 10, 1924; but it was not at all difficult to determine that the achenes were neither lenticular nor trigonous. In all other respects as well the type specimens are identical with the plants here listed. The four cotypes and two other sheets were examined: one from Laggan, Rocky Mountains, June 28, 1904, and the other from near International Boundary between Kettle and Columbia Rivers, June 9, 1902; both collected by J. M. Macoun and verified by Mr. Mackenzie. These six sheets contained fifteen plants, of which four showed mature tetramerous achenes; four others had 4 stigmas; four were too immature to display either stigma or achene characters, and three plants were without inflorescence.

As a result of this review of the literature and specimens, the authors feel that *Carex concinnoides* Mackenzie should have its description emended to read *stigmas four, achene tetragonous*. As this one species differs so fundamentally from the remaining thousand or so species, a new subgenus and section are here proposed to receive it.

#### ALTERICAREX subgen. nov.

*Eucarex similans*, sed ramis stigmatis 4, nucibus 4-angulosis.

Resembling the subgenus *Eucarex*, but having four stigmas, and a four-angled achene. It includes the section *Tetragonae*, and the species *Carex concinnoides* of western North America.

#### TETRAGONAE sect. nov.

Stolonifera, spiculis masculis 1 terminalibus, spiculis femineis 1-2 pro-pinquis, utriculis pubescentibus stipitatis ellipticis brevirostratis truncatis, stigmatibus 4, nucibus stipitatis 4-angulosis.

Stoloniferous; staminate spike 1, terminal; pistillate spikes 1-2, approximate; perigynia stipitate, elliptical, pubescent, short-beaked, the beak truncate at tip; stigmas four; achene stipitate at base, 4-angled.

The section contains one species, *C. concinnoides* Mackenzie of western North America.

CAREX CONCINNOIDES Mackenzie, Bull. Torrey Bot. Club. 33: 440, 441. 1906. Although placed in a different subgenus and section, it roughly resembles *C. concinna* R. Br., and more closely *C. Richardsonii* R. Br., both of which are in the *Digitatae*. Mr. Mackenzie in the original publication of the species, cited above, gives in key form a number of characters helpful in separating these three species. *Carex concinnoides* comes into bloom in Washington and Idaho in late April or early May, when the culms are only

a few centimeters high and are much overtopped by the leaves. The culms elongate rapidly, often attaining a length of 40 cm., and by June, when the fruits are ripe, the culms arch over like a blackberry stem and rest the spikes on the ground. In early July the fruits are practically all shed. The leaf blades are scabrous on the margins.

Type locality: Montana: Columbia Falls, *R. S. Williams*, June 7 and July 28, 1893.

Specimens examined:

British Columbia: border of Emerald Lake, altitude 4300 ft., Selkirk Mts., *C. H. Shaw* 77a, July 1, 1904; Deer Park, Lower Arrow Lake, *John Macoun* 56, June 6, 1890; Spence's Bridge, *John Macoun* 63, June 20, 1876; without locality, *John Macoun* 247, June 20, 1875; near Laggan, altitude 6000 ft., *C. O. Rosendahl* 1135, Aug. 24, 1902; Laggan, Rocky Mountains, *John Macoun* 64,020, June 28, 1904; near International Boundary between Kettle and Columbia Rivers, *J. M. Macoun* 63,296, July 9, 1902; mountain top, valley of Fraser River, *J. Macoun* 44, May 21, 1875.

Washington: in moss, Twisp, *Velma Batie*, May 24, 1922; Ellensburg, *Kirk Whited* 304, April 24, 1897; Roslyn, *Kirk Whited* 304, April 24, 1897; open woods near Gardiner Caves, Pend Oreille Co., *H. St. John* 3253, June 24, 1924; open woods, Slate Creek, 6 miles north of Metaline Falls, *H. St. John* 3252, June 17, 1924; Fan Lake, Pend Oreille Co., *Carl H. Spiegelberg*, also *Roderick Sprague* 715, May 8, 1923; bank of Pend Oreille River, Newport, *Carl H. Spiegelberg*, May 5, 1923; moist open wood near Penrith, *Roderick Sprague* 714, May 3, 1923; Mica Peak, *W. N. Suksdorf* 8841, July 10, 1916; moist wooded land, north slope near top of Kamiak, *Charles S. Parker* 420, May 28, 1922; abundant on wooded north side of Kamiak Butte, *H. St. John* 3251, May 3, 1924; moist ravine, Blue Mts., *Robert M. Horner* 209, April 24, 1897, and *Robert M. Horner* 648, April 4, 1897. It has been observed, but not collected, by *H. St. John* between Nespelem and Omak Lake; and at 23 Mile Creek, San Poil River valley.

Idaho: Moscow Mt., *Leroy Abrams* 636, May 1906; *Earl Ihrig*, May 13, 1922; *Virgil Argo*, May 14, 1922; *H. St. John* 3250, May 4, 1924; and *H. St. John* 3249, June 10, 1923.

California: Red Mts., Mendocino Co., *H. N. Bolander* 6478, in 1866.

Alberta: Banff, *John Macoun* 7464, June 26, 1891, and *M. A. Barber* 92, June 25, 1903; Pipestone Creek, *John Macoun* 64,021, June 29, 1904; Lake Agnes, altitude 6900 ft., *Ezra Brainerd* 110, Aug. 17, 1897.

Montana: Columbia Falls, *R. S. Williams*, June 7 and July 28, 1893; Columbia Falls, *R. S. Williams* 953, June 7, 1893.

## EXPLANATION OF PLATE VII

The illustrations were prepared partly from fresh, partly from dried, material by Mr. and Mrs. St. John.

- A. Habit view of mature *Carex concinnoides*.  $\times \frac{2}{3}$ .
- B. Distal end of achene.  $\times 13$ .
- C. Lateral view of achene.  $\times 13$ .
- D. Lateral view of perigynium.  $\times 13$ .
- E. Young pistillate flower, showing four stigmas.  $\times 6\frac{1}{2}$ .
- F. Scale.  $\times 6\frac{1}{2}$ .
- G. Young inflorescence.  $\times 6\frac{1}{2}$ .



ST. JOHN AND PARKER: CAREX





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## A PRELIMINARY SKETCH OF THE PLANT REGIONS OF OREGON II. THE CASCADE MOUNTAINS AND EASTWARD

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In a paper published in the preceding issue of this JOURNAL, the author discussed the six plant regions of that part of Oregon lying west of the Cascade Mountains. We shall now consider the remainder of the state, that is, the Cascade Mountains together with what is usually spoken of as Eastern Oregon.

### CASCADE REGION

This region, as here discussed, includes, briefly, the Cascade Mountains of Oregon, extending the full length of the state from north to south. The Paulina Mountains form a small "island" on the eastern side. On the west it passes gradually into the Willamette and Rogue-Umpqua regions, as previously stated, except at the extreme southern end of the state, where it meets the region last considered. On the eastern side the Cascades drop rather abruptly to the general level of the eastern Oregon plateau, that is, to approximately 1300 M. The boundaries are least definite at the southern end, where the mountains fall away to a mere divide with moderate slopes descending very gradually into adjoining regions.

Except for a few areas too small to be of significance in the present discussion, the surface of the whole Cascade region consists of lavas of different ages. Particularly along the western side, they are old enough to have acquired a heavy mantle of soil, the formation of which has been accelerated by the heavier precipitation of this portion. The higher ridges and peaks to the eastward are much newer, some of the flows being so recent as to have acquired scarcely a vestige of soil.

The fact that the highest part of the region is near its eastern border, thus giving a much longer westward slope, is of great significance, since it causes, over nearly the whole area, a heavy precipitation by the interception of the moisture brought from the Pacific by the westerly winds as they rise gradually to the higher altitudes. The precipitation ranges from 2000 mm.,

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at moderate altitudes on the western slope toward the north, to 1000 mm. at a corresponding altitude on the same slope near the southern end of the area, but there are great local variations. The snowfall along the western border is relatively slight; at high altitudes it is extremely heavy.

Hitherto in this discussion we have made no mention of life zones, for the reason that each of the regions thus far considered lies almost wholly within one zone, or else the zonal characters are intermediate or variously mixed. Thus, according to accepted criteria, the Northern Coast Mountain and Willamette Valley regions are practically pure Transition; the Northern Coast region unites features of the Transition and Canadian; the Rogue-Umpqua is a patchwork of Transition and Upper Sonoran; the Southern Coast Mountain and Siskiyou is predominately Transition with perhaps small patches of Upper Sonoran and a few points rising into Canadian, and the Southern Coast is Transition. With the Cascade region the case is somewhat different. We are here dealing with a set of factors that are relatively uniform throughout a large area, with the exception of altitude and of those conditions immediately correlated with it. The vegetation thus assumes a characteristic zonal distribution dependent primarily upon altitude, though influenced locally to a certain extent by direction of slope, soil conditions, etc. We may then with advantage apply the life-zone concept and terminology to our discussion of this region.

The lowest zone represented is the Transition. Its upper limit rises to a maximum altitude of about 1300 M.; it is considerably lower toward the north and on cooler slopes and in narrow valleys. In the gorge of the Columbia River it is scarcely represented at all. On the west it passes of course gradually into the Transition of the Willamette and Rogue-Umpqua regions. On the eastern side it is extremely narrow.

Originally almost the whole of this area was heavily forested, but fires and lumbering operations have devastated large sections of it. The character of the forest was much like that of the Coast Mountain forest, but there were fairly well marked differences. It was less dense and uniform, with a much more copious undergrowth of shrubs and herbaceous plants. The most abundant conifers were *Abies grandis*, *Pseudotsuga taxifolia*, *Thuja plicata*, and *Tsuga heterophylla*.

The distinction between this zone of the Cascades and the regions to the east and west is too vague to make it advisable to present a list of characteristic species. Such distinguishable features as it possesses are due to the general facies of the vegetation rather than to the particular assemblage of species.

The Canadian zone has a much better definition than the Transition, the latter passing into it more or less gradually as we ascend. The Canadian reaches a maximum altitude of perhaps a little under 2200 M., dropping lower, of course, as we go northward. Much the greater part of it lies between 1500 and 1800 M.

The surface is more broken, and the slopes are steeper, than those of the Transition. Deep canyons and narrow valleys are frequent, often carrying the zone to lower levels, and rough talus slopes of loose lava are abundant. Waterfalls, wet cliffs, and small bogs are also plentiful.

Originally this zone, like the preceding, was mostly covered with forest, and has suffered less at the hand of man; open, stony tracts are frequent, however, especially toward the southern end of the region. The most abundant conifers are *Pinus monticola*, *P. contorta*, *Abies nobilis*, *A. grandis*, *Pseudotsuga taxifolia*, and *Tsuga heterophylla*. The undergrowth of shrubs is less abundant in species and less dense than in the Transition.

The following are typical or very abundant forms of this zone: *Phegopteris dryopteris*, *Pinus monticola*, *P. contorta*, *Abies nobilis*, *A. concolor*, *A. amabilis*, *A. grandis*, *Pseudotsuga taxifolia*, *Tsuga heterophylla*, *Picea Engelmanni*, *Hierochloa macrophylla*, *Cinna latifolia*, *Pleuropogon refractus*, *Erythronium montanum*, *E. parviflorum*, *Xerophyllum tenax*, *Streptopus curvipes*, *S. amplexifolius*, *Clintonia uniflora*, *Iris tenuis*, *Cephalanthera Austinae*, *Listera caurina*, *L. convallarioides*, *Montia asarifolia*, *Isopyrum Hallii*, *Cimicifuga elata*, *Trautvitteria grandis*, *Delphinium trolliifolium*, *Corydalis Scouleri*, *Ribes bracteosum*, *Saxifraga Mertensiana*, *Tiarella unifoliata*, *Rubus nivalis*, *Pyrus sitchensis*, *Pachistima myrsinites*, *Acer Douglasii*, *Epilobium oregonense*, *E. fastigiatum*, *Cornus canadensis*, *Chimaphila Menziesii*, *Pyrola secunda*, *Pterospora andromedea*, *Menziesia ferruginea*, *Vaccinium macrophyllum*, *V. parvifolium*, *Polemonium carneum*, *Romanzoffia sitchensis*, *Mertensia bella*, *Pentstemon diffusus*, *Chelone nemorosa*, *Pedicularis bracteosus*, *Viburnum pauciflorum*, *Erigeron pacificus*, *E. amplifolius*, *Aster modestus*, *Raillardella argentea*, *Senecio triangularis*, *Antennaria racemosa*, *Hieracium cinereum*.

The Hudsonian zone includes only a very limited area on the higher peaks and ridges. It is also more restricted in altitudinal range than the Canadian, extending from the upper limit of the latter to the tree limit, usually between 2300 and 3000 M. During winter it is covered with snow to a great depth, which does not disappear before late June.

The most characteristic topographical features are barren lava ridges with steep sides often covered with large boulders, dry gravelly slopes, and small bogs and meadows.

The most abundant and characteristic tree of this zone is *Tsuga Mertensiana*. It often occurs unmixed with other forms in extremely dense stands, almost completely excluding any undergrowth of herbaceous species. *Pinus albicaulis* is second in abundance, and *Chamaecyparis nootkatensis*, *Larix Lyallii*, *Abies lasiocarpa*, and *A. magnifica* are of local occurrence. Tall shrubs are few. The bogs and meadows are remarkable for their brilliant floral display.

While the majority of Hudsonian plants of this region have a considerable north and south range in the Cascades of Oregon, a number of species have

been recorded only from the extreme north or extreme south; the difference in this respect, however, is too slight to make farther discrimination necessary. The following are among the most characteristic species: *Chamaecyparis nootkatensis*, *Pinus albicaulis*, *Larix Lyallii*, *Abies magnifica*, *A. lasiocarpa*, *Tsuga Mertensiana*, *Agrostis Rossae*, *Danthonia intermedia*, *Deschampsia atropurpurea*, *Festuca viridula*, *Bromus Suksdorfii*, *Carex ablata*, *C. vesicaria*, *C. Mertensii*, *C. oregana*, *C. spectabilis*, *C. Preslii*, *C. Jonesii*, *C. nervina*, *C. teneraeformis*, *C. integra*, *C. Rossii*, *Salix commutata*, *Polygonum shastensis*, *P. minimum*, *P. bistortoides*, *P. phytolaccaefolium*, *Eriogonum umbellatum*, *Lewisia triphylla*, *Spraguea multiceps*, *Anemone hudsoniana*, *Ranunculus alismellus*, *R. Eschscholtzii*, *R. Gormani*, *Caltha leptosepala*, *C. biflora*, *Aquilegia truncata*, *Aconitum Howellii*, *Arabis Drummondii*, *Sedum divergens*, *Ribes Howellii*, *R. erythrocarpum*, *Saxifraga bronchialis*, *S. Bongardi*, *S. arguta*, *Mitella pentandra*, *M. Breweri*, *Spiraea densiflora*, *Rubus lasiococcus*, *Potentilla flabellifolia*, *P. cascadiensis*, *Pyrus occidentalis*, *Epilobium alpinum*, *E. fastigiatum*, *Cassiope Mertensiana*, *Phyllodoce empetritiformis*, *Kalmia glauca microphylla*, *Rhododendron albiflorum*, *Gaultheria humifusa*, *Dodecatheon Jeffreyi*, *Douglasia laevigata*, *Phlox Douglasii*, *Polemonium humile*, *Mimulus Lewisii*, *M. primuloides*, *Pedicularis groenlandica surrecta*, *P. bracteata*, *Castilleja Suksdorfii*, *Valeriana sitchensis*, *Agoseris barbellulata*, *Erigeron salsuginosus*, *Aster Engelmanni*, *A. Covillei*, *A. foliaceus*, *Senecio subnudus*.

The Arctic-Alpine zone is much more restricted than the Hudsonian, embracing only a few isolated peaks that rise above the limit of forest trees. Most of even this small area is scarcely fit for plant life, owing to lack of soil. There are occasional depressions containing bogs or small streams, dry gravelly slopes, and crevices among boulders where scanty soil has accumulated; otherwise there are only barren peaks and ledges and tumbled masses of lava rock. The ground is bare of snow for from two to three months.

Aside from the absence of erect trees, the distinction between this zone and the Hudsonian is vague. There is usually a "Krummholz" of *Pinus albicaulis* and *Tsuga Mertensiana* bordering the upper limit of the Hudsonian. Numerous herbaceous species are about equally divided between the two zones. The following forms are among the most characteristically Arctic-Alpine: *Agrostis humilis*, *Trisetum spicatum*, *Poa arctica*, *P. alpina*, *P. paddensis*, *Sitanion glabrum*, *Carex gymnoclada*, *C. illota*, *C. nigricans*, *C. straminiformis*, *C. vernacula*, *C. Breweri*, *Juncus Parryi*, *J. Mertensianus*, *Oxyria digyna*, *Eriogonum pyrolaeifolium coryphaeum*, *Polygonum Newberryi*, *Silene Suksdorfii*, *Arenaria capillaris*, *Anemone occidentalis*, *Draba aureola*, *Cardamine bellidifolia*, *Arabis platysperma*, *Leptarrhenia amplexifolia*, *Saxifraga Tolmiei*, *Lutkea pectinata*, *Sibbaldia procumbens*, *Epilobium anagallidifolium*, *Phyllodoce glandulifera*, *Phlox diffusa*, *Gilia debilis*, *Pentstemon Menziesii*, *Veronica Wormskioldii*, *Pedicularis contorta*, *Castilleja*

*oreopola*, *Agoseris alpestris*, *Hieracium gracile*, *Erigeron compositus trifidus*, *Aster alpigenus*, *A. Andersoni*, *Hulsea nana*, *Antennaria media*.

#### EASTERN OREGON REGION

This is nearly equal in area to all the other regions combined, comprising as it does all the state east of the Cascade Mountains, except the Blue Mountain region and two or three small "islands." It presents considerable diversity of topography, soil, climate, etc., with corresponding diversity of vegetation, but we have found it impracticable completely to segregate the several sections geographically. It consists mainly of a plateau with a general elevation of about 1200 M. but the northern end gradually sinking away toward the Columbia River, where the elevation is very slight. The Stein Mountains may be considered as an outlying "island" of the Blue Mountain region, or possibly belonging with the Rocky Mountains, while the Paulina Mountains we have considered with the Cascade region.

The drainage of the region has an important bearing on the character of the vegetation. The northern and most of the eastern and western portions are drained by streams belonging to the Columbia River system, while a small area in the southwest has its drainage exit by way of Upper Klamath Lake and Klamath River. The remainder, comprising between a third and a half of the total area, belongs to the Great Basin, the scanty streams of which empty into shallow lakes without outlets.

The geological formation is relatively simple, nearly the whole area being composed of lavas of various forms, all of which are alkaline. About the lakes and in dry lake beds of the Great Basin section are numerous large tracts of Quaternary gravel, sand, and silt, all of volcanic origin. The lower parts of the Klamath Lake basin are of similar character. The rest consists mostly of lavas of the great Tertiary and Quaternary inundations and of the products of their disintegration untransported by water.

The depth of the soil mantling the lava is highly variable, ranging from almost nothing to hundreds of feet. In some places it is extremely fine volcanic dust, in others very coarse, depending largely upon the nature of the original deposit. The Quaternary lake-bed deposits above mentioned are nearly all more or less alkaline, often strongly so, as a result of the decomposition products of the alkaline lavas having drained into them from higher surrounding territory.

The annual precipitation for several points within the region is as follows: Umatilla, 200 mm.; Heppner, 350 mm.; Dayville, 300 mm.; Bend, 375 mm.; Burns, 300 mm.; Vale, 250 mm.; Prineville, 225 mm.; Paisley, 200 mm.; Klamath Falls, 325 mm. A large proportion of this falls as snow. This low precipitation coupled with the high elevation produces great day and night variations of temperature, so that over most of the region frosts may occur at any time during the year.

While we find it impossible to subdivide this large territory into distinct

regions, relying upon such criteria as were employed for distinguishing those regions already considered, we find it equally impossible to treat it as a unit; we have therefore chosen a middle course and shall discuss it under the headings of several areas. These areas are not sufficiently distinct in their boundaries to be adequately represented on our regional map, yet each has its own unquestionable characteristics which demand recognition. We may designate and limit them as follows:

*Columbia River Area.* This includes a narrow strip of territory along the Columbia, beginning to the eastward where the Columbia first becomes the Oregon-Washington boundary and following the stream down to where it begins to break through the Cascades. It is broadest, perhaps ten to fifteen miles in width, at its upper end, but narrows below to the immediate shores of the river.

*Yellow Pine Area.* This name is suggested by Professor Piper's "Yellow Pine Forest," the name given to the corresponding area in eastern Washington. It comprises a zone sometimes very narrow, sometimes many miles in width, fringing the eastern base of the Cascades and the lower slopes of the Blue Mountains all round. It also includes the higher slopes and summits of various short mountain chains lying within the Great Basin territory, but does not occur in the Stein Mountains.

*Bunch-grass Area.* This name also is borrowed from Professor Piper. It lies mainly between the Columbia River area on the north and the Yellow Pine area fringing the western extension of the Blue Mountains on the south, its southern limit to the west of the latter being ill defined. It also includes most of the territory in the valleys of the Powder and Grande Ronde Rivers, and a small part of the northeast corner of the State.

*Lake Area.* This might be called the Great Basin area, but the name chosen seems more appropriate, since its boundaries do not quite coincide with those of the Great Basin in Oregon. A line drawn from the southern boundary of the state northward to the Stein Mountains and along their western base, thence northward to the latitude of Burns, then westward to the foot of the Paulina Mountains and southwestward across the southern end of Klamath Lake to the California-Oregon line, will approximately define the area.

*Sagebrush Area.* This name is far from satisfactory, but it is chosen for want of a better. It includes all sections of the Eastern Oregon region not coming within the previously defined areas.

We may now consider separately each of the foregoing divisions.

### Columbia River Area

This small tract has several well marked physical characteristics. The precipitation is very low, especially to the eastward. The soil is exceptionally sandy, the sand largely brought by winds from the Columbia River and forming widespread drifts and dunes. During most of the summer very

strong westerly winds prevail with high temperatures; it is thus one of the most arid sections of the state. In consequence of these conditions, the vegetation is mostly very dwarfed. The common sagebrush, *Artemisia tridentata*, abounds, but rarely reaches normal size; the same is true of other shrubs that usually accompany it, such as *Purshia tridentata* and species of *Chrysothamnus*. The following species are especially characteristic of the area: *Marsilia vestita*, *Oryzopsis hymenoides*, *Festuca octoflora*, *Agropyron subvillosum*, *Elymus flavescens*, *E. arenicola*, *Salix amygdaloides*, *Celtis Douglasii*, *Rumex venosus*, *Polygonum majus*, *Salsola Kali tenuifolia*, *Corispermum hyssopifolium*, *Abronia mellifera*, *Lesquerella Douglasii*, *Erysimum occidentale*, *Cleome lutea*, *Purshia tridentata*, *Psoralea lanceolata scabra*, *Asiragalus succumbens*, *A. podocarpus*, *A. Spaldingii*, *Erodium cicutarium*, *Euphorbia serpyllifolia*, *Opuntia polyacantha*, *Gaura parviflora*, *Anogra pallida*, *Leptotaenia salmoniflora*, *Pteryxia terebinthina*, *Centaureum exaltatum*, *Verbena bracteosa*, *Plantago Purshii*, *Xanthium Wootoni*, *Gaertneria acanthicarpa*, *Grindelia nana columbiana*, *Chrysopsis villosa*, *Chrysothamnus viscidiflorus*, *Solidago serotina salebrosa*, *S. occidentalis*, *Coreopsis Atkinsoniana*, *Gaillardia aristata*, *Artemisia dracunculoides*, *A. tridentata*, *Cirsium undulatum*.

### Yellow Pine Area

This comprises mainly the most elevated territory of the arid section of the Transition zone of Oregon, usually passing above into the Canadian. Naturally the differences between the two divisions, namely, that associated with the Cascades and that associated with the Blue Mountains, are considerable, and they might well be treated separately.

The soil of this area is nearly everywhere light and sandy with a minimum of humus. The precipitation averages perhaps a little higher than in the unforested portions of the region with a somewhat larger proportion of snow.

As the name implies, the western yellow pine, *Pinus ponderosa*, is the dominant species of the area. It never forms a dense growth and usually has few other conifers associated with it; along the upper limit, however, it mingles with *Pseudotsuga taxifolia*, *Abies grandis*, *Pinus contorta*, and, in the Blue Mountain section, *Larix occidentalis*. Its lower border, especially along the Cascades, is often fringed with *Juniperus occidentalis*. Shrubby and herbaceous vegetation is remarkably scant, both in individuals and in species. A yellow pine forest has usually the appearance of having been carefully cleared of all undergrowth. The following list includes the most characteristic species. Many of them are confined mainly or wholly to one or the other of the two sections.

*Pinus ponderosa*, *Stipa occidentalis*, *S. minor*, *Calamagrostis rubescens*, *Poa Olneyae*, *Carex petasata*, *Disporum majus*, *Betula microphylla*, *Alnus tenuifolia*, *Arceuthobium campylopodum*, *Eriogonum heracleoides*, *E. spergu-*



*linum*, *Arenaria lateriflora*, *A. congesta*, *A. glabrescens*, *Clematis hirsutissima*, *Berberis repens*, *Arabis Holboellii*, *Sedum Douglasii*, *Heuchera glabella*, *Ribes inerme*, *R. petiolare*, *Opulaster pauciflorus*, *Spiraea Menziesii*, *Rubus strigosus*, *Potentilla Blaschkeana*, *P. Nuttallii*, *Horkelia fusca*, *Fragaria platypetala*, *Geum Oregonense*, *G. ciliatum*, *Crataegus columbiana*, *Prunus emarginata*, *Lupinus laxiflorus*, *Trifolium longipes*, *Astragalus Hoodianus*, *Lotus Douglasii*, *Lathyrus bijugatus Sandbergii*, *Linum micranthum*, *Ceanothus velutinus*, *Shepherdia canadensis*, *Pyrola picta*, *P. elata*, *Pterospora andromedea*, *Gentiana affinis*, *Gilia Harknessii*, *Mertensia nutans*, *Cryptantha affinis*, *Lithospermum ruderales*, *Pentstemon variabilis*, *P. venustus*, *Mimulus nanus*, *Pedicularis semibarbatas*, *Chamaesaracha nana*, *Galium asperrimum*, *G. multiflorum*, *Kelloggia galioides*, *Phyloria lactucina*, *Agoseris glauca*, *Ericameria Bloomeri*, *Aster conspicuus*, *A. Fremonti*, *Senecio cymbalarioides*, *Antennaria Geyeri*, *A. luzuloides*, *A. rosea*, *Cirsium Drummondii*.

### Bunch-grass Area

This section comprises the lower portion of the Arid Transition zone in Oregon. With little above the average precipitation for the region, it is by far the most important agriculturally of all that part of the state lying east of the Cascades. This is due primarily to the character of the soil, which over most of the area is a very deep, fine silt loam mostly of volcanic origin. Locally, as in the Grande Ronde valley, it is alluvial. Nearly everywhere it is remarkably retentive of moisture. There are no extensive lava exposures. The surface is mostly hilly; the hills are frequently bold and high, but more often lower with moderate slopes; however, there are large areas of quite level ground.

This is the great wheat-growing section of Oregon; as the name indicates, however, the dominant native vegetation was the various species of bunch-grass, especially *Agropyron spicatum*, *Festuca idahoensis*, and *Poa Sandbergii*. These have now largely disappeared through cultivation and close pasturage. The area is almost wholly destitute of native trees, and shrubby vegetation is plentiful only along streams. Introduced species here form a far larger part of the wild vegetation than in any other section of the state east of the Cascades.

The following are among the most characteristic species of this area: *Poa Sandbergii*, *Festuca idahoensis*, *Bromus rubens*, *B. tectorum*, *B. hordeaceus*, *B. brizaeformis*, *Hordeum murinum*, *Agropyron spicatum*, *Brodiaea Douglasii*, *Calochortus macrocarpus*, *Iris missouriensis*, *Eriogonum niveum*, *Delphinium simplex*, *Platyspermum scapigerum*, *Draba verna*, *Lesquerella Cusickii*, *Sisymbrium alissimum*, *Lepidium perfoliatum*, *L. dictyotum*, *Cleome platycarpa*, *Lupinus leucophyllus*, *L. canescens*, *L. ornatus*, *Astragalus stenophyllus*, *A. reventus*, *A. misellus*, *Lathyrus coriaceus*, *Petalostemum ovatum*, *Geranium viscosissimum*, *Sidalcea oregana*, *Cactus viviparus*, *Clarkia pulchella*, *Lomatium Piperi*, *Frasera nitida*, *Phlox canescens*, *Gilia Bolanderi*,

*Oreocarya celosioides*, *Scutellaria angustifolia*, *Pentstemon erianthus*, *Castilleja lutescens*, *Orthocarpus luteus*, *Ptiloria tenuifolia*, *Crepis occidentalis*, *C. gracilis*, *C. barbigera*, *Brickellia oblongifolia*, *Grindelia nana*, *Ericameria nana*, *Aplopappus carthamoides*, *Erigeron filifolius*, *Boltonia occidentalis*, *Balsamorhiza sagittata*, *Helianthella Douglasii*, *Artemisia rigida*, *Cirsium palousense*.

### Sagebrush Area

Much the greater part of the section thus named consists of irregular hills often of considerable height but seldom very steep. There are, however, wide tracts of approximately level territory. Many of the streams flow through steep-walled canyons, their walls above being formed of nearly vertical rim-rock. The soil is extremely varied, though nearly all of volcanic origin. In the valleys, where it has gradually accumulated as alluvium, it is of excellent quality, though the area is limited. There are also considerable sections having a uniformly fine, deep soil, which, when capable of irrigation, become valuable agricultural lands. For the most part, however, there is mingled with the finer constituents of the soil a greater or less quantity of fragmentary lava, often with a more continuous layer not far below the surface. Those areas where the surface lava is very abundant are known as "scab-lands."

*Artemisia tridentata* is of course the most characteristic species of this area. While it occurs, often in abundance, in other areas, it attains nowhere else in Oregon such a uniform distribution and typical development. Several other shrubs of the same general habit are also abundant, and in the more elevated sections *Juniperus occidentalis* is plentiful. The total number of herbaceous species and under-shrubs is very large; it thus becomes difficult to select a list that shall include the most important and characteristic forms of all that go to make up the total of the vegetation, without making it of inordinate length. We would include the following: *Juniperus occidentalis*, *Poa nevadensis*, *Stipa Thurberiana*, *Festuca octoflora*, *F. pacifica*, *Bromus tectorum*, *Elymus condensatus*, *Carex Douglasii*, *Allium Cusickii*, *Leucocrinum montanum*, *Zigadenus paniculatus*, *Eriogonum angulosum*, *E. proliferum*, *E. microthecum*, *E. vimineum*, *E. Baileyi*, *E. Douglasii*, *Chorizanthe Watsoni*, *Grayia spinosa*, *Lewisia rediviva*, *Arenaria Franklinii*, *Delphinium bicolor*, *Parrya Menziesii*, *Arabis subpinnatifida*, *A. puberula*, *A. arcuata*, *Thelypodium integrifolium*, *Thelypodium milleflorum*, *Smelowskia Fremonti*, *Schoenocrambe linifolia*, *Cercocarpus ledifolius*, *Purshia tridentata*, *Amelanchier utahensis*, *Peraphyllum ramosissimum*, *Trifolium macrocephalum*, *T. Plummeri*, *T. Beckwithii*, *Astragalus leucocystis*, *A. lentiginosus*, *A. obscurus*, *A. Beckwithii*, *Rhus toxicodendron*, *Sphaeralcea Munroana*, *Viola trinervata*, *V. Beckwithii*, *V. vallicola*, *Mentzelia albicaulis*, *Anogra trichocalyx*, *Sphaerostigma andinum*, *S. Hulgardi*, *S. Boothii*, *S. alyssoides*, *Gayophytum ramosissimum*, *Lomatium Grayi*, *L. Donnellii*, *Leptotaenia multifida*, *Phlox longifolia*, *P. rigida*, *Gilia pungens Hookeri*, *G. filifolia*, *G. inconspicua*,

*Navarretia Breweri*, *Lappula diffusa*, *L. cupulata*, *L. Cusickii*, *Piptocalyx circumscissus*, *Pectocarya setosa*, *Cryptantha pteryocarya*, *Allocarya mollis*, *Pentstemon glaber*, *P. collinus*, *Mimulus nanus*, *M. Cusickii*, *Castilleja pallescens*, *Cordylanthus ramosus*, *Solanum triflorum*, *Valerianella macrocera*, *Psiloria paniculata*, *P. exigua*, *Crepis acuminata*, *Chrysothamnus nauseosus*, *C. viscidiflorus*, *C. puberulus*, *Aplopappus lanuginosus*, *A. stenophyllus*, *Townsendia florifer*, *Erigeron hispidissimus*, *E. poliospermus*, *E. linearis*, *E. divergens*, *Aster scopulorum*, *Blepharipappus glandulosus*, *Helianthus Cusickii*, *Eriophyllum multiflorum*, *Chaenactis Douglasii*, *Artemisia tridentata*, *A. arbuscula*, *Tetradymia canescens*, *Senecio Howellii*.

### Lake Area

The climatic differences between this and the area last considered are not sufficient to cause any appreciable difference in the character of the flora; practically the only factor, therefore, to be taken into account in distinguishing the two from each other is the difference in soil. The soil of the present area is relatively very poor, with few good deposits of fine volcanic material free from coarse fragments of lava, except for several limited tracts about Upper Klamath and Goose Lakes, where the deposits are largely alluvial. There are, it is true, about the shrunken lakes, swamps, and old lake beds, extensive deposits of fine material, but these are hard, heavy, and more or less strongly impregnated with alkali from the decomposition of lavas. There being no drainage outlet except for a small part of the territory adjacent to Upper Klamath Lake, the alkali accumulates in the depressions. The area of this alkaline land has been greatly increased by the gradual drying up of the numerous large lakes that once occupied this territory to a mere fraction of their former size. Many are now tule swamps; others are too strongly alkaline to support vegetable life.

On the whole, the conditions on the higher lands of this area are more severe than those of the Sagebrush area, due to the much poorer soil conditions. While this results in a well marked difference in the general character of the flora and also in the species, it is in the alkaline sections that the most strongly marked characteristics of the area appear. Of the numerous halophytic forms occurring here, a number are found as well, though mostly in less abundance, in other alkaline tracts of eastern Oregon, such as certain parts of Powder River valley.

In the following list of the most abundant and the most characteristic species, we are obliged to include several that were given for the preceding area on account of their dominance over large sections. The great majority are scarce or unknown in the state outside of this area, except for the halophytic forms above mentioned.

*Triglochin palustris*, *Damsonium californicum*, *Distichlis spicata*, *Puccinellia Lemmonii*, *P. Nuttalliana*, *P. distans*, *Agropyron caninum*, *A. lanceolatum*, *A. Smithii*, *Scirpus nevadensis*, *S. Olneyae*, *S. occidentalis*, *S.*

*validus*, *Oxytheca dendroides*, *Sarcobatus vermiculatus*, *Suaeda intermedia*, *Kochia americana*, *Eurotia lanata*, *Atriplex confertifolia*, *A. pusilla*, *Chenopodium salinum*, *Monolepis pusilla*, *Nitrophila occidentalis*, *Calyptidium roseum*, *Spergularia diandra bracteata*, *Loeflingia squarrosa*, *Thelypodium brachycarpum*, *T. flexuosum*, *Stanleya viridiflora*, *Roripa columbiae*, *Lepidium montanum*, *Prunus subcordata*, *Lupinus subvexus transmontanus*, *Astragalus salinus*, *A. malacus*, *A. pterocarpus*, *Oenothera Hookeri*, *Chylisma scapoides*, *Lomatium nevadense*, *Rhysopterus plurijugus*, *Glaux maritima obtusifolia*, *Heliotropium curassavicum*, *Plagiobothrys Harknessii*, *Ailocarya jucunda*, *Scutellaria nana*, *Madronella glauca*, *Antirrhinum Kingii*, *Pentstemon Kingii*, *Nicotiana attenuata*, *Laurentia carnosula*, *Lygodesmia spinosa*, *Eatonella nivea*, *Glyptopleura marginata*, *Malacothrix Torreyi*, *Iva axillaris*, *Brickellia microphylla*, *Chrysothamnus viscidiflorus*, *Aplopappus curvatus*, *Brachyactis frondosa*, *Wyethia mollis*, *Chaenactis xantiana integrifolia*, *Tanacetum potentilloides*, *Artemisia tridentata*, *A. arbuscula*, *A. spinescens*, *Tetradymia spinosa*.

#### BLUE MOUNTAIN REGION

In this are included the Blue and Wallowa mountains with their various subdivisions, spurs, and outlying ridges. Altitude is, of course, the chief factor in determining the boundaries. Being bordered nearly all round by the Yellow Pine area of the region last considered, the upper limit of the range of *Pinus ponderosa* may be taken as the boundary. Naturally, this is not sharply defined.

The region is wholly mountainous, the highest elevations rivaling the highest parts of the Cascades. Geologically, more than half of the total area is of the same formation as that prevalent over most of the Eastern Oregon region, namely, Tertiary lavas. The remainder, including the more elevated portions, is mainly of older rock, metamorphosed sedimentary formations of Jurassic and Triassic age and slates, schists, and gneisses of Paleozoic. The most characteristic flora of the region occurs on these older and more elevated formations.

The average precipitation is about 625 mm., a large proportion of which falls as snow. In a few places at high altitudes it exceeds 1000 mm. As is the case with the rest of the state east of the Cascades, the latter intercept most of the moisture from the Pacific. The total area retaining perennial snow is extremely small.

While this region includes territory falling within all of the higher life zones, that of the Hudsonian and the Arctic is so limited in extent that it seems unnecessary to treat them separately.

As already indicated, *Pinus ponderosa* of the Yellow Pine area mingles with *Abies grandis*, *Pseudotsuga taxifolia*, *Pinus contorta*, and *Larix occidentalis* along the boundaries of the Blue Mountain region. *Pinus contorta* mostly dominates the Canadian zone, often forming extensive pure stands

of moderate density. Aside from these areas there are few sections where the forest is not more or less open, a fact due to the rather low precipitation. There is nowhere anything to compare with the magnificent forests of the Cascades. Near the upper limit of trees *Pinus albicaulis* is plentiful, but always forming a scattered growth. There is often a moderately dense undergrowth of shrubs, such as *Pyrus sitchensis* and *Ceanothus velutinus*. At the higher altitudes, wide, open tracts are common.

A large proportion of the species of this region also occur in the Cascades, but the flora contains likewise a considerable Rocky Mountain element with a number of endemic species. Professor Piper, in his "Flora of Washington," gives a list of characteristic Sierra forms that are found in the Blue Mountains but are not known from the Cascades. This latter fact, however, is in many cases probably due to our incomplete knowledge of the flora of the east slope of the Cascades, since several of the species listed have been found there since the publication of the above-named work.

The following are among the most abundant and characteristic species of this region: *Pinus albicaulis*, *P. contorta*, *Larix occidentalis*, *Abies lasiocarpa*, *A. grandis*, *Pseudotsuga taxifolia*, *Tsuga Mertensiana*, *Picea Engelmanni*, *Agrostis idahoensis*, *Danthonia intermedia*, *Poa Bolanderi*, *Bromus Suksdorfii*, *Carex atratiformis*, *C. atosquama*, *C. festivella*, *C. Lewisii*, *C. phaeocephala*, *C. stricticulmis*, *C. hystricina*, *C. Tolmiei*, *C. vernacula*, *Allium macrum*, *A. collinum*, *Camassia Cusickii*, *Calochortus apiculatus*, *Zigadenus elegans*, *Salix Barclayi*, *S. reticulata*, *Eriogonum strictum*, *E. Piperi*, *Lewisia nevadensis*, *Claytonia megarrhiza*, *Silene scaposa*, *Clematis columbiana*, *Thalictrum sparsiflorum*, *Ranunculus populago*, *R. ciliosus*, *Delphinium scopulorum subalpinum*, *D. cyanoreios*, *Corydalis Cusickii*, *Draba stenoloba*, *D. Cusickii*, *D. alpina*, *D. glacialis*, *D. nitida*, *D. Lemmonii*, *Thlaspi glaucum*, *Saxifraga odontophylla*, *Heuchera ovalifolia*, *Parnassia fimbriata*, *Ribes lentum*, *Potentilla brevifolia*, *Pyrus sitchensis*, *Lupinus sulphureus*, *L. Sabinii*, *Aragallus Cusickii*, *Astragalus Hookerianus*, *Lathyrus rigidus*, *Ceanothus velutinus*, *Rhamnus alnifolia*, *Gayophytum pumilum*, *Osmorhiza occidentalis*, *Lomatium Cusickii*, *Ligusticum tenuifolium*, *L. Leibergeri*, *Pyrola chlorantha*, *Vaccinium scoparium*, *V. macrophyllum*, *Primula Cusickiana*, *Androsace septentrionalis*, *Gentiana simplex*, *Frasera speciosa*, *F. Cusickii*, *Madronella nervosa*, *Pentstemon fruticosus*, *Veronica Cusickii*, *Castilleja indecora*, *C. rubida*, *C. rustica*, *C. Cusickii*, *Lonicera utahensis*, *Sambucus melanocarpa*, *Aplopappus hirtus*, *A. Greenii*, *Townsendia alpigena*, *Erigeron membranaceus*, *E. armeriaefolius*, *E. Bloomeri*, *E. Coulteri*, *Aster integrifolius*, *A. Cusickii*, *Rudbeckia occidentalis*, *Senecio hydrophiloides*, *S. condensatus*, *Saussurea americana*.

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# THE EFFECT OF SELENIUM COMPOUNDS UPON GROWTH AND GERMINATION IN PLANTS<sup>1</sup>

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Sulfur is a necessary constituent of plants. Since selenium and tellurium according to the periodic law belong to the same group as sulfur, it becomes of interest to determine their occurrence in plant tissue and their possibility of replacing sulfur in plant metabolism. It is very likely that these elements and their compounds are present in soil, and it is therefore of considerable importance to observe their effect upon germination and growth.

Selenium was discovered by Berzelius in 1817. He recovered it from the deposit in the lead chambers of a sulfuric acid factory in Gripsholm, Sweden. He named it selenium (Greek, moon) on account of its resemblance to tellurium (Greek, earth) discovered a short time before. Selenium is widely distributed in small quantities. It rarely occurs in the free state, and is often associated with native sulfur and native sulfides. It also occurs in meteoric iron. The usual commercial sources of selenium are the residues of the sulfuric acid factory. When iron pyrites is burned, the volatilized selenium and selenium dioxid are deposited in the flues of the Glover tower and in the chambers.

Selenium is an amorphous or crystalline solid; tasteless, odorless, and insoluble in water. It melts at 217° C. and boils at 675° C. The vapor is dark yellow and possesses an odor of putrid horse-radish. When suddenly cooled, the vapor condenses to a fine, brick-red powder (flowers of selenium). Selenium, like sulfur, appears in allotropic forms. One of these, metallic selenium, a gray or black solid, has a metallic appearance and conducts electricity. Its electrical conductivity is peculiarly affected by light and heat, increasing when heated and especially when exposed to light.

In its chemical properties, selenium closely resembles tellurium. It is somewhat less negative than sulfur, and has valencies of two, four, and six. Chemically it is inactive, until heated. It burns with a reddish-blue flame to form selenium dioxid. It unites readily with hydrogen, phosphorus, the halogens, and with metals to form selenids. Selenium can replace sulfur in alums and in organic compounds.

Selenium and tellurium differ from sulfur in the ease with which their compounds are reduced to the free element. Compounds of sulfur, like

<sup>1</sup> This work was done in the Department of Biological Chemistry, College of Physicians and Surgeons, Columbia University. The author takes great pleasure in acknowledging the valuable advice and stimulation of Professor William J. Gies.

sulfides and sulfites, undergo oxidation in the biologic organism to form non-toxic sulfates; while corresponding compounds of selenium and tellurium undergo reduction, in part, to the free non-toxic element, and escape, in part, with the expired air in the form of a volatile organic selenid or tellurid of characteristic odor. Selenium and tellurium also differ from sulfur in the greater toxicity of their compounds.

C. Knop in 1885 examined a number of inorganic compounds with reference to their effect on plant growth. Among these were compounds of silver, gold, platinum, boron, iron, chromium, molybdenum, tungsten, cadmium, thorium, lead, bismuth, arsenic, tellurium, and selenium. His experiments with compounds of the last two concern us particularly. He found that the plants treated with solutions which contained 0.005 percent or 0.01 percent of the neutralized tellurous or telluric acid grew as well as the control. They developed green leaves, but never blossomed. Tellurous acid proved more toxic than telluric acid.

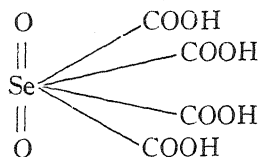
Similar experiments with solutions of 0.005 percent and of 0.01 percent selenious and selenic acids, subsequently neutralized, showed at once poisonous effects. In the strong concentrations of both neutralized acids he observed after two days wilting and withering of the roots. The roots of the weaker concentrations followed the same course, but four or five days later. In comparison with neutralized tellurous acid or telluric acid, the selenium compounds proved more toxic. He recovered selenium and tellurium in the ashes from the upper portions of the treated plants.

A few years earlier, Cameron observed the effects of potassium selenate. He reported one experiment, a description of which follows: A sod, two feet deep, three feet in length, and one foot wide, was taken from a field in which a crop of leguminous plants was just peeping over the ground. The sod was placed in a box, and one-half of the plants were watered twice a week with a dilute solution of potassium selenate ( $K_2SeO_4$ ). The total quantity of the salt used within four weeks amounted to twenty grams. No difference could be perceived between the plants to which the potassium selenate had been applied and those to which it had not. Cameron concluded that selenic acid in small quantities does not injure plants. From his wording it seems that he did not appreciate the difference between a salt and an acid and that biologic findings holding true for an acid (presence of hydrogen ions) may not be applicable to its salts. That the selenium compounds were taken up and retained by the plants, he proved by analysis. The plants were carefully washed in order to separate any traces of adhering selenate, partially dried, and boiled in strong nitric acid until thoroughly oxidized. The solution was evaporated to dryness and the residue was dissolved with dilute hydrochloric acid. This solution, concentrated and mixed with a saturated solution of sulfurous acid, assumed a deep blood-red color due to the formation by reduction of elemental selenium.

Recently Gassmann has claimed that selenium in minute quantities is found in plants. He believes it to be present in the octavalent condition in



the form of a compound in which one molecule of selenium dioxid reacts with two molecules of oxalic acid:



The findings of Gassmann, however, have been severely criticized by Fritsch, who has not been able to confirm them.

In order to determine the effect of selenium compounds on germination and growth of plants, solutions of selenium dioxid (selenious acid) and selenic acid in distilled water were made up in various dilutions: 0.0001 percent, 0.001 percent, 0.01 percent, 0.025 percent, 0.05 percent, 0.1 percent, 0.2 percent, 0.25 percent, 0.4 percent, 0.5 percent, and 1 percent. For sodium selenite, sodium selenate ( $\text{Na}_2\text{SeO}_4 \cdot 10\text{H}_2\text{O}$ ), and potassium selenocyanid ( $\text{KSeCN}$ ), besides the strengths mentioned, solutions of 0.75 percent, 2.5 percent, and 5 percent were also prepared. Control tests were made with distilled water and with tap water.

#### EFFECT ON GERMINATION OF SEEDS

##### Experiments with Lupin Seeds

White lupins were allowed to soak for twenty-four hours in selenium acid and salt solutions of concentrations ranging from 0.0001 percent to 1 percent or 2.5 percent, and then allowed to germinate. The results of the experiments follow.

With selenium dioxid, germination took place in these concentrations: 0.0001 percent and 0.001 percent. Only one out of five seeds succeeded in germinating in the 0.01-percent solution. In sodium-selenite solutions, evidence of germination of all the seeds used in the experiment was found in the 0.0001-percent and in the 0.001-percent dilutions; but only one out of five seeds germinated in 0.01-percent and in 0.025-percent solution. Germination with selenic acid took place only in concentrations of 0.0001 percent and 0.001 percent. Sodium selenate ( $\text{Na}_2\text{SeO}_4 \cdot 10\text{H}_2\text{O}$ ) was not as toxic as the other compounds, for seeds germinated in the following dilutions: 0.0001 percent, 0.001 percent, 0.01 percent, 0.025 percent, 0.05 percent. Potassium selenocyanid gave positive results for germination with dilutions of 0.0001 percent, 0.001 percent, 0.01 percent, and 0.025 percent.

It is to be remarked that in all cases in which there was germination there was no reduction of the selenium compounds, but in the higher concentrations in which no germination took place the cotyledons assumed, after days, a light red or brick-red tint owing to the formation of elemental selenium. The reduction is probably to be ascribed to the activity of micro-organisms.

### Experiments with Timothy Seedlings

Timothy seedlings were soaked in various concentrations of selenium solutions for a period of twenty-four hours. The solutions were then decanted and the seedlings were thoroughly washed several times with distilled water. They were afterwards transferred to petri dishes having strips of filter paper at the bottom. The paper disks were covered with glass beakers and sprayed daily with distilled water, or with the various selenium solutions.

Germination was observed in the seedlings soaked with 0.0001-percent, 0.001-percent, and 0.01-percent selenium dioxide. After two weeks, no growth had taken place even in the seedlings that were treated with the 0.001-percent solution of selenium dioxide. In the sodium-selenite solutions, germination and even growth had occurred in seedlings treated with the 0.0001-percent, 0.001-percent, 0.01-percent, 0.025-percent, and 0.05-percent solutions. The abundance in growth was inversely proportional to the concentrations of the solutions in which the seedlings had been soaked. In no case was the growth as good as in the control.

With selenic acid, germination took place in seedlings treated with the 0.0001-percent, 0.001-percent, and 0.01-percent solutions. Growth occurred only in 0.0001-percent selenic acid, but the height of the grass was one half that in the control. Sodium selenate proved by far the least toxic. Growth almost as good as in the control occurred up to the 0.2-percent concentration.

Timothy seedlings with potassium selenocyanid germinated when soaked in concentrations of 0.0001 percent, 0.001 percent, 0.01 percent, 0.025 percent, and 0.05 percent. Growth occurred only in the seeds treated with the 0.0001-percent solution, but the height of the grass blades was one fourth that in the control.

## EFFECT ON THE GROWTH OF PLANTS

### Experiments with White Lupins

The white lupins selected were soaked in distilled water for twenty-four hours. They were then grown in wet moss which had been washed clear of all visible impurities. At the end of two days they were removed from the moss, carefully wiped on a smooth cloth, and the shells were peeled. A mark made with India ink was placed 15 mm. from the tip of each seedling. The bean was then carefully speared upon a glass rod, supported by a cork plate partially covering the beaker. It was suspended in such a manner that the rod only was immersed in the liquid. The dilutions were: 2.5 percent, 0.75 percent, 0.2 percent, 0.1 percent, 0.05 percent, 0.025 percent, 0.01 percent, 0.001 percent, 0.0001 percent. Control tests were run with distilled water and with tap water.

The observations made daily were the following: the amount of chlorophyll-formation, the length of the root, the growth of side roots, the extent

of fungous and protozoan growth in the solutions, the reduction of the selenium compounds.

Some of the observations are recorded in tabular form.

TABLE 1. *Selenium Dioxid* ( $\text{SeO}_2$ )

Date	Controls		Concentrations				
	Tap Water, Growth in mm.	Distilled, Growth in mm.	0.0001 %, Growth in mm.	0.001 %, Growth in mm.	0.01 %, Growth in mm.	0.05 %, Growth in mm.	0.025 %, Growth in mm.
Aug. 19...	34	26.5	35	30	18	17	15
Aug. 20...	45	29	45	35	Shriveled	Shriveled	Shriveled
Aug. 21...	68	29	50	43			

Judging from the control kept with distilled water, selenium dioxid in concentrations of 0.0001 percent and 0.001 percent accelerated the growth of roots. Higher concentrations up to and including 0.05 percent gave meager growth. Still higher concentrations did not permit growth at all. Growth of side roots in the 0.0001-percent solution was as good as in the control, while in the 0.001-percent selenium dioxid the number of side roots was less than in the control or in the 0.0001-percent solution. No side roots were formed in the 0.025-percent solution. Tuft-growth and chlorophyll-formation were quite marked in the 0.0001-percent and the 0.001-percent solutions, but not in the higher concentrations. Growth in the 0.0001-percent selenium-dioxid solution continued as long as in the distilled-water control, while the plant in the 0.001-percent selenium-dioxid solution died earlier.

TABLE 2. *Sodium Selenite* ( $\text{Na}_2\text{SeO}_3$ )

Date	Controls		Concentrations						
	Tap Water, Growth in mm.	Dis- tilled, Growth in mm.	0.001 %, Growth in mm.	0.01 %, Growth in mm.	0.025 %, Growth in mm.	0.05 %, Growth in mm.	0.1 %, Growth in mm.	0.2 %, Growth in mm.	0.75 %, Growth in mm.
July 25.....	20	18	22	18	16	16	16	15.2	15
July 26.....	24	20	26	18	17	17	17	15	With- ered
July 28.....	36	25	31	18	17	17	18	Shriv- eled	
July 29.....	40	25	27	Shriv- eled	Shriv- eled	Shriv- eled	Shriv- eled		
July 30.....	43	26	27						

A 0.001-percent solution of sodium selenite had evidently little effect on growth of roots when we compare the results with the distilled-water control. Growth was less, however, than that found in the tap water. Higher concentrations inhibited the growth of roots, and no growth at all was observed in concentrations above 0.1 percent. An exuberant development of side roots was noticed in the 0.001-percent solution; there were fewer side roots in the 0.01-percent solution and none in the 0.025-percent solution.

Tuft-growth and chlorophyll-formation occurred in the 0.001-percent solution, but not in the others. Withered roots were colored brick-red by the reduced sodium selenite.

Very recently, Turina has treated plants with selenium and tellurium compounds and has followed the path of reduction of these compounds by the presence of deposits of the free granules of free selenium or tellurium.

TABLE 3. *Selenic Acid* ( $\text{H}_2\text{SeO}_4$ )

Date	Controls		Concentrations			
	Tap Water, Growth in mm.	Distilled, Growth in mm.	0.0001 %, Growth in mm.	0.001 %, Growth in mm.	0.01 %, Growth in mm.	0.025 %, Growth in mm.
Aug. 11..	29	20	43	24	16	15
Aug. 12..	30	21	50	30	18	17
Aug. 14..	34	23	55	37	18	17

A 0.0001-percent and a 0.001-percent selenic acid brought about better root growth than did the tap water or distilled water in the controls, though side rootlets were not as abundant as in the controls. The increase in growth with very small concentrations of selenium dioxid and of selenic acid seems to be in accord with the findings of Promsy, who observed that germination and growth were accelerated by dilute solutions of citric, malic, tartaric, oxalic, acetic, hydrochloric, and sulfuric acids. Tuft-growth and chlorophyll-formation were quite marked. There was no growth of roots in concentrations above 0.001 percent, as well as no side roots and no chlorophyl- or tuft-development.

TABLE 4. *Sodium Selenate* ( $\text{Na}_2\text{SeO}_4 \cdot 10\text{H}_2\text{O}$ )

Date	Controls		Concentrations				
	Tap Water, Growth in mm.	Distilled, Growth in mm.	0.01 %, Growth in mm.	0.025 %, Growth in mm.	0.05 %, Growth in mm.	0.1 %, Growth in mm.	0.25 %, Growth in mm.
Aug. 11..	29	20	20	18.5	15	15	15
Aug. 12..	30	21	21	19	17	17	16
Aug. 14..	34	23	22	18	17	17	17

Measurements of root lengths could not be taken in the 0.0001-percent and in the 0.001-percent solutions, as the root tips had accidentally been broken off. One could, however, observe an abundance of side growth and the development of chlorophyl and of tufts. Side roots were to be seen even in the 0.25-percent solution, the profuseness of growth being inversely proportional to the concentration of the sodium-selenate solution.

From the results obtained with sodium selenate it appears that concentrations of 0.01 percent (0.005 percent on an anhydrous basis) are not

toxic to plant growth. Knop, however, claimed that neutralized selenic acid in concentrations of 0.005 percent and 0.01 percent is toxic, although the same concentrations of neutralized sulfuric, telluric, and arsenic acids were without effect.

Comparing the results on the plant activity of the selenite ion (selenious acid and sodium selenite) with those of the selenate ion (selenic acid and sodium selenate), we find the former more toxic than the latter. Stoklasa also found selenites more inimical than selenates. He found, however, that treating plants with radioactive water greatly reduced the toxicity of selenites partly by their conversion to selenates.

TABLE 5. *Potassium Selenocyanid* (KCNSe)

Date	Controls		Concentrations			
	Tap Water, Growth in mm.	Distilled, Growth in mm.	0.0001 %, Growth in mm.	0.001 %, Growth in mm.	0.01 %, Growth in mm.	0.025 %, Growth in mm.
Aug. 11..	29	20	28.5	44	23	15
Aug. 12..	30	21	29	54	20	15
Aug. 14..	34	23	30	55	Shriveled	Shriveled

Concentrations of potassium selenocyanid from 0.0001 percent to 0.001 percent did not inhibit growth of the main and side roots, tuft-development, or chlorophyll-formation, all of which were as vigorous as in the controls. Concentrations greater than 0.01 percent were completely toxic. The solutions showed no growth of fungi or of protozoa.

Compared with the experiments of Kahn, potassium selenocyanid is as toxic to plant life as potassium sulfocyanid.

### Experiments with Timothy Seedlings

Pieces of filter paper were allowed to soak in distilled water for several minutes, then put in a flat petri dish and sprinkled evenly with timothy-grass seedlings. The paper was then covered with a glass beaker and sprayed twice daily with distilled water until the grass blades developed to a height of about one half inch. Each grass plot was thereafter watered twice daily with several drops of a definite strength of a selenium acid or salt solution. Several controls were kept with distilled water. Concentrations varying from 0.0001 percent to 5 percent were used.

The injurious effects of the selenium compounds were noticed within two days. The blades no longer increased in size, they became greenish yellow in color, and after ten days shriveled and blackened. Mold-growth was observed in all the plots in which no growth had taken place. These findings hold true for concentrations greater than 0.01 percent in the case of selenious acid, selenic acid, sodium selenite, and potassium selenocyanid, and in concentrations greater than 0.1 percent in the case of crystalline sodium selenate.

Growth was obtained in the 0.0001-percent and 0.001-percent selenium-dioxid solutions, although the height of the grass blades was less than in the control. With sodium selenite there was poor growth in the 0.001-percent solution and very slight growth in the others. Sodium selenate did not prove to be as toxic as the other compounds, since growth could be observed in the 0.0001-percent, 0.001-percent, 0.01-percent, and even in the 0.025-percent, 0.5-percent, and 0.1-percent concentrations. Growth in the last two dilutions mentioned was, however, very poor. Potassium selenocyanid in concentrations of 0.0001 percent and 0.001 percent permitted the growth of timothy seedlings; higher concentrations did not.

A notable feature of these experiments was the appearance of a ring of red selenium, which followed the outline of the filter paper. These rings were observed with selenium dioxid, sodium selenite, selenic acid, and potassium selenocyanid, but not with sodium selenate. The decompositions were probably due to the action of micro-organisms. It is interesting to note in this connection that Levine has observed the formation of free, brick-red selenium when bacteria are grown in media containing selenium dioxid, selenic acid, and sodium selenite. No formation of free selenium was observed with media containing sodium selenate.

#### SUMMARY

The selenium compounds studied, when in concentrations of 0.01 percent and over are exceedingly inimical to germination as well as to growth of plants.

The compounds may be arranged as follows in the order of diminishing toxicity: selenious acid, selenic acid, sodium selenite, sodium selenate, and potassium selenocyanid. This order agrees well with that obtained by the author in experiments on animals.

Increased growth has been observed with very dilute solutions (0.0001 percent, 0.001 percent) of selenium dioxid (selenious acid) and of selenic acid.

Selenium compounds are taken up and retained by plants (Cameron, Knop).

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## PRUINOSE BRANCHLETS AND *SALIX LEMMONII* BEBB

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When Bebb<sup>1</sup> described his *Salix lemmonii* and *S. austinae*, he discussed the color of the branchlets in both cases but made no mention of a pruinose bloom or covering in either case. Twelve years later, when he reviewed<sup>2</sup> the species of willows presented in the Flora of California, he again did not mention pruinose twigs in either case.

### THE OCCURRENCE OF PRUINOSE BRANCHLETS

Several species of *Salix* have more or less pruinose twigs. Among North American species, *S. irrorata* And., *S. bella* Piper, *S. pellita* And., *S. subcoerulea* Piper, and *S. geyeriana* And. are well known examples, belonging to three different sections of the genus. Among European species, *S. daphnoides* Vill. is a conspicuous representative. This phenomenon occurs widely in the plant world. Cabbage leaves, the fruits of plums and blueberries, the stems and leaf-sheaths of rye, maize, and cultivated sorghums, and many related wild grasses, especially in the tribes Andropogoneae, Maydeae, and Paniceae, will come readily to mind.

The conditions under which this powdery exudate is formed, and its permanence after initial appearance, are not well understood. This question becomes important when the presence and absence of a pruinose bloom are used as diagnostic characters by botanists. Some years ago the senior writer collected a flowering specimen of *Salix daphnoides* in a willow holt. When collected and placed in press, the brownish-black twigs were entirely free from any pruinose covering. When the drying specimen was examined in press a day or two later, the twigs were entirely covered with the white bloom. A twig character which comes and goes apparently would have little taxonomic value.

In 1921, the senior writer received a collection of willows from Mr. Kirk Whited of Redmond, Oregon, the junior author of this paper and a long-time student of the plants of the Pacific Northwest. Among these *Salices* were two specimens which were determined as *S. lemmonii* Bebb. One of these (446b) comprised twigs collected on February 12, May 4, and June 4, 1921, representing bursting buds, flowers and young leaves, and full-grown leaves, respectively. The one-year- and two-year-old branchlets and the old bud-scales are conspicuously pruinose in collections of all dates, while

<sup>1</sup> Bebb, M. S., in Watson, S., Bot. Calif. 2: 88. 1879.

<sup>2</sup> Bebb, M. S. Notes on North American willows VI. A review of the willows of California. Bot. Gaz. 16: 106. 1891.



the young shoots of the season on the specimen of June 4 are only faintly pruinose. The second specimen (446c) was collected on May 17 and consists of flowers and unfolding leaves of both sexes. Here again the one-year branchlets, mostly orange yellow, and the 2-year branchlets, becoming brownish, are markedly pruinose. Older portions are only faintly so, and seasonal growth, which was just beginning, shows no bloom at all.

The abundant occurrence of the bloom on twigs one and two years old, and the complete or nearly complete lack of it on twigs of the season and those more than 2 years old, opened a very interesting question. Accordingly, the junior writer undertook to collect specimens from the same plant at intervals throughout the year, with such observations on the effect of rain, snow, drought, etc., on the pruinose bloom as might serve to throw light on the problem of its occurrence.

During the winter of 1921-1922, and the summer and autumn of 1922, the junior writer made a collection of willows which contained thirteen numbers of *Salix lemmonii* Bebb. Of these, eight numbers represented material collected from the same plant on two different dates, while two of the eight had been collected on three different dates. The collection dates ranged from November 1, 1921, to September 5, 1922.

It was felt that this material would repay critical study because (1) it contained suites of specimens from the same plants, (2) it was all collected within the range of a few miles, and (3) all the plants had grown under fairly uniform conditions, mostly along irrigation ditches, at elevations of about 3,000 to 4,500 feet.

The first number (503-2) was collected on April 12 (bursting buds) and May 3, 1922 (flowers), while mature foliage from the same plants had been collected on November 1, 1921. The one- and two-year twigs were very pruinose on April 12, the one-year-old shoots of May 3 show only the faintest trace of bloom, while the one- and two-year twigs were moderately pruinose on November 1.

The portion of a second number (503-21) collected on April 5, 1922, when the buds were bursting or the aments were partly expanded, shows heavy bloom on the yellowish yearling branchlets of the previous season and a light deposit on two-year-old branchlets. A portion collected from the same plant on September 5, 1922, shows a light bloom on the yellowish seasonal twigs, while a portion collected on November 1, 1921, has a heavy bloom both on the fully mature seasonal twigs and on those of the previous year, and on the bud-scales of both. A third number (503-18), consisting of a leafy sprout 7 dm. long collected November 2, 1921, shows heavy bloom on the terminal fourth, light bloom on the next lower fourth, and none at all on the lower half.

On flowering specimens collected on April 23 (503-50), the bloom on the long orange to brownish shoots is faint but evident. Numbers 503-54 and 503-56 contain flowering specimens collected on April 30. The short,

divaricate, one- and two-year-old orange branchlets are densely pruinose in the former and faintly so in the latter, while a single longer brownish-black shoot in the latter is absolutely without bloom. In Numbers 503-60 and 503-61, the flowering specimens, collected on May 1, are long, straight, brownish-black shoots 35 cm. long, the former evidently pruinose but the latter showing only a faint bloom on the lower third and on bud-scales higher up. In all these five numbers, except 503-56, the flowering specimens are accompanied by mature foliage collected from the same plants on September 2, 3, and 4. All except number 503-54 show well developed bloom on both one- and two-year-old orange or brown branchlets and bud-scales. Number 503-54 is faintly pruinose on yearling and seasonal branchlets.

Of still other numbers, young fruiting shoots were collected on May 26 (551 and 551*a*) and May 29 (no. G of Tumalo trip). Shoots bearing mature fruits were collected on June 14 (551*a*) and 13 (551*c*), and shoots bearing fully-expanded leaves on June 14 (551, 551*a*) and June 13 (551*c*). The one- and two-year twigs collected on May 26 are moderately pruinose in 551 and heavily so in 551*a*. The bloom is conspicuous on the 60-cm. female shoot of no. G, while it is only moderately to faintly present on the male shoot of equal length (both May 29).

On the older fruiting shoots of 551*a* and 551*c* (June 13, 14) the bloom is evident, but not as heavy as on the one- and two-year twigs of the foliage specimens collected on the same days from the same plants of 551, 551*a*, and 551*c*. The leafy seasonal shoots, which already had been developed in 1922 to a length of from 5 to 14 cm., are not yet pruinose, however, though the bloom is beginning to appear on the bases of these shoots on no. 551.

In order to make the results more evident to the reader, they are presented in condensed form in table I. A study of this table shows, in general, that, under the Redmond conditions, (1) specimens collected in early spring before anthesis usually are heavily covered with the pruinose bloom; (2) in the late spring, during the flowering period, the branches tend to lose this bloom and it may disappear completely from some; (3) in early summer, when fruit is ripening and leaves have become full-sized, there is a tendency for the branchlets to regain their heavy bloom, though in some cases it may be faint, and new growth does not show it at all; and (4) material collected in autumn, from September 2 to November 2, usually shows heavy bloom again, though sprouts may show little. In general, the long blackish sprouts of the previous season show much less of the bloom at any season than do the short, divaricate, yellowish branchlets which terminate the older branches. So far as noted, no bloom occurs on any branchlets after their third season.

Three suites of similar specimens collected in 1923, but not included in the tabulation, show essentially the same characteristics.

Fourteen additional specimens collected in 1923 do not alter in any way the conclusions drawn from observations in 1921 and 1922. Numbers 1, 2,

and 3 were collected in the vicinity of Redmond at intervals from April to July, inclusive, and all show an abundant pruinose covering. Numbers 4, 5, 9, 13, 19, and 30 were collected in May, June, and July at various points along the Columbia Southern irrigation ditch several miles to the southwest of Redmond and at considerably higher elevations.

The junior writer states that in nature, on *Salix lemmonii*, the pruinose deposit, when it first appears, is firm and is not affected by the heaviest rains, nor can it be removed by the tongue or lips until it has begun to disintegrate.

TABLE 1. Quantity of pruinose bloom on branchlets of *Salix lemmonii* Bebb from near Redmond, Oregon, when collected from the same plants at different seasons of the years 1921 and 1922

(Symbols: X = heavy bloom, + = moderately light bloom, - = faint bloom, and o = no bloom)

Number of Plant	Plants Bearing			
	Buds	Flowers	Fruit	Autumn Foliage
	Feb. 12-Apr. 12	Apr. 23-May 17	May 26-June 14	Sept. 2-Nov. 2
446b.....	X	X	X	
446c.....			X	
503-2.....	+	-		+ - o
503-18...				X + - o
503-21...	X -			X + - o
503-50...				X -
503-54...		X		
503-56...		- o		
503-60...		+ - o		X
503-61...		- o		X
551.....			+ -	
551a.....			+ o	
551c.....			+ o	
G.....			+ -	

An examination of specimens from other parts of Oregon, from California, and from Nevada, in the senior writer's herbarium and the National Herbarium, shows that specimens from Oregon east of the Cascade Mountains generally are densely pruinose, those from southwestern Oregon and Siskiyou County, California, are lightly pruinose, while those from the Sierra Nevada of California and from western Nevada are only faintly or not at all pruinose. Most of the specimens from the Sierra Nevada, however, were collected when in flower or fruit, at which stage of development the least bloom would be expected. Cusick's number 1836, from northeastern Oregon, collected on April 18, May 9, and in September, shows the same general seasonal expression of the pruinose bloom as do the Whited specimens.

In the case of *S. lemmonii*, the bloom is most abundantly deposited on plants in the northern part of the range, while in the closely-related *S. geyeriana* the plants from Wyoming, Colorado, and Utah are densely pruinose and those from Idaho, Oregon, and Washington less so, the north-western variety *meleina* Henry lacking the bloom entirely.

If the exudation of the powdery bloom is a physiological safeguard against too great or too rapid desiccation, the semi-arid conditions of Oregon east of the Cascades and in the Siskiyou district might account for its relative abundance there, and the more humid conditions on the west slope of the Sierras might explain its rare occurrence there. In eastern Oregon, the relative lack of bloom in spring and early summer could be correlated with lack of its production in that period or with its washing off by spring showers.

#### THE POSITION OF *SALIX AUSTINAE* BEBB

In his discussion of *Salix lemmonii* Bebb and *S. austinae* Bebb, Schneider<sup>3</sup> (pp. 75-81) retains the latter as a variety of *lemmonii* chiefly on the ground that *S. austinae* has pruinose twigs while those of *lemmonii* are not pruinose or glaucous. Speaking of the branchlets, Schneider says (*l. c.*, p. 79) that "those of the male specimen are hardly pruinose while the glaucous bloom is rather conspicuous on the branchlets of the female specimen which are of the same color."

In the National Herbarium is a specimen (sheet 667040) collected by Mrs. R. M. Austin (1081) in American Valley (probably in Placer County. C. R. B.), California, in April, 1897, and labelled "*lemmoni*." It is annotated by Schneider "! C. Schn. 1919," but is not cited by him in the paper referred to. Of the five slender yellow flowering branchlets, three (two female and one male) show faint traces of bloom while the two remaining female twigs do not. In the light of all these findings it seems hardly possible to maintain *austinae* as a variety based on this character.

Schneider also has established *S. alaxensis longistylis* (Rydb.) Schn. n. comb.,<sup>4</sup> based chiefly on pruinose twigs (p. 225). The writer has not studied the material representing this form.

#### REDESCRIPTION OF *SALIX LEMMONII*

*Salix lemmonii* Bebb in Watson Bot. Calif. 2: 88. 1879.

*Salix lemmonii austinae* (Bebb) Schneider, Jour. Arnold Arboretum 2: 79. 1920.

*Salix austinae* Bebb in Watson Bot. Calif. 2: 88. 1879.

Shrub 1.5 to 3 or 4 m. tall; bark on the usually shining, one- or two-year-old branchlets orange yellow to golden brown to dark brown or purplish

<sup>3</sup> Schneider, C. Notes on American willows X. Jour. Arnold Arboretum 2: 65-90. 1920.

<sup>4</sup> Schneider, C. Notes on North American willows VIII. Jour. Arnold Arboretum 1: 211-232. 1920.

black, varying apparently with age, exposure, and rate of growth; long, slender sprout shoots always dark-colored, seasonal branchlets often thinly villous with silvery hairs, the one- and two-year twigs usually more or less pruinose, especially in the northern or drier portions of its range; bud-scales ovoid or broadly lanceolate, 6-11 mm. long, colored as the branchlets, glabrate or glabrous; stipules usually obsolete or minute and deciduous, occasionally conspicuous on leafy sprouts, and then lanceolate or semi-ovate, acute, 6-13 mm. long, serrate; petioles 3-10 mm. long, often thinly pilose; blades narrowly oblanceolate or lanceolate, or elliptic-lanceolate, broadest about the middle, apex acute or the lower obtusish, base acute or obtuse, entire or remotely denticulate, 3-7 or 8  $\times$  0.7-1.5 or 1.8 cm., common sizes being 4  $\times$  0.7, 5  $\times$  1.0-1.2, 6  $\times$  1.2-1.4, 7  $\times$  1.3-1.8, or, on sprouts, 8-10 cm.  $\times$  1.5-1.8 cm., bright green above, glaucescent to nearly glaucous beneath, especially when full-grown, the recently expanded often thinly pubescent on both surfaces, especially beneath, plane, or the veins on old leaves somewhat evident, midrib yellowish beneath.

Aments coetaneous, leafy-pedunculate, ascending; peduncles 0.5-1 cm. long, silky-pubescent (as the rachis), bearing 2-4 leaves 1-2 cm. long and often deciduous later. Masculine aments cylindrical, densely flowered, 1-3 cm. long, about 1 cm. broad; scales obovate or obovate-oblong, usually obtuse, black, thinly long-villous (the dark scale readily visible); stamens 2, filaments free or rarely united for one third or less of their length, the lower half pilose; anthers yellow; gland 1. Feminine aments 3-4 or 5 cm. long in anthesis, 4-6 or 7 cm. long and 1.5 cm. broad in fruit; scales as described above; ovary in anthesis about 5 mm. long; capsule narrowly ellipsoid-rostrate, 5-8 mm. long, thinly to densely pubescent; pedicel 1-2 mm. long, pubescent; style 0.5-1 mm. long, entire or nearly so; stigmas 2, entire or bifid, about 0.5 mm. long; gland 1, filiform, yellowish, about 1 mm. long.

Flowers and expanding foliage in early to middle April and fruit in late April and early May at lower elevations, and from 2 to 3 weeks later at the higher elevations.

Mountain streams and ditches from Payette County, Idaho, westward in Oregon to the Cascades and south to Jackson and Klamath Counties, Oregon; Washoe and Ormsby Counties, Nevada; and San Bernardino County, California, at elevations from 3,500 to 10,000 feet.

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# THE OCCURRENCE OF PURPLE BACTERIA AS SYMBIONTS OF A LICHEN

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*Chiodecton sanguineum* (Sw.) Waino is a very conspicuous and attractive lichen which occurs quite abundantly in several parts of Florida; the writer has also observed it in some sections of the island of Cuba. As far as I am aware, it is far more abundant in the damp hammocks, especially in the low hammocks, than in the dry regions covered with pine and xerophytic shrubs. I found it abundantly upon stems of *Quercus virginiana*; also, although less abundantly, on *Magnolia glauca*, *M. grandiflora*, *Acer rubrum*, and *Nyssa biflora*. I have never observed it on any of the conifers.

Specimens found in South America have been described by Waino (11); he gives no description of microscopic details.

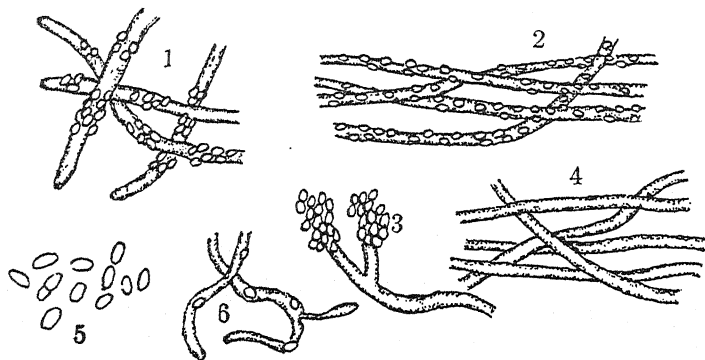
It seems that in this section of the United States fruiting bodies have not yet been found. Also, Professor Charles C. Plitt of Baltimore, who is well acquainted with lichens, never collected fruiting specimens in Florida, Jamaica, or Cuba. Mr. W. W. Diehl of Washington, D. C., stated to me that in the U. S. National Herbarium some individuals appear to have fruiting bodies.

The flat, crust-like lichen, which may reach a diameter of from half a centimeter to two decimeters, is roundish, oval, or uneven. Many appear gray to bluish-gray in color with a deep red margin which is practically never absent. Between the grayish parts the red color may appear in streaks, in spots, or scattered almost like dust. Although the upper part, with the exception of the margin, may be entirely gray, the lower surface is deep red, as may easily be observed when the plants are carefully peeled from the stem upon which they grow.

When the lichen has been wet for a few days the red color may change to violet. When examined under the high power of the microscope, the mycelium of the fungus is easily visible. It is at once evident that the hyphae are surrounded by one-celled organisms which are especially crowded toward the apices of the hyphae (text fig. 3), as may often be observed toward the periphery of the lichen body. These one-celled organisms are colored and are the cause of the red color of *Chiodecton*. Sporadically we find them amidst the gray part, but they are common upon the lower surface.

The red symbionts never occur in threads or in chains, except directly after division, since they soon become free. They are oval, and vary from 1.5 to 2 microns in length (text fig. 5) when the lichen has been dry

for some time; upon being moistened they vary from 0.5 to 2 microns. The hyphae of the fungus have a width of 3 to 5 microns; they are transparent when alive, but when dead they sometimes have a pink color, probably due to the absorption of the red pigment of the one-celled organisms.



TEXT FIG. 1.—Ends of hyphae on the lower surface of the lichen covered with purple bacteria. TEXT FIG. 2.—Hyphae of the lower surface covered with purple bacteria. TEXT FIG. 3.—Purple bacteria crowded at the tops of hyphae toward the periphery of the lichen. TEXT FIG. 4.—Hyphae of the fungus in pure culture. TEXT FIG. 5.—Single cells of *Rhodobacterium lichenophorum* from a pure culture. TEXT FIG. 6.—Hyphae and bacteria from a hanging-drop culture.

I find that the red pigment of the colored cells dissolves at once in methyl alcohol, more slowly in ethyl and propyl alcohols, and hardly at all in amyl alcohol. It also dissolves fairly well in ether, and is dissolved in benzol after a few days. When to a methyl-alcohol solution some osmic tetroxid is added, the solution soon becomes wine red, then brownish, and finally greenish yellow. If, however, sulfuric or hydrochloric acid is added, it becomes orange red. The color is not changed by the addition of sodium hydroxid. A dark red solution in methyl alcohol becomes yellow and then colorless after the addition of iodine. When the red pigment is extracted from the cells, the latter show very small, transparent granules.

I isolated the red organisms and tried to grow them in hanging-drop cultures in water, but without success. It is easy, however, to cultivate them in the culture medium used by Molisch (9) for purple bacteria, namely:

Tap water.....	1000 grams
Agar.....	10 grams
Pepton.....	5 grams
Dextrin.....	5 grams

To this I added a decoction of *Chiodecton sanguineum*. For this purpose, 1 gram of the lichen was boiled in 10 cc. of water, and 2 grams of the extract were added to the nutrient solution. The first cultures were made on November 2, 1923. With a loop of platinum wire the organisms were

placed on the medium, and, after 8 days, in all three petri dishes growth was easily visible on account of the presence of the red color. Cultures developed also in three of seven test tubes. Hanging drop cultures of the same mixture were also made, all of which were successful. The organisms do not liquefy agar or develop gases in pure cultures. The colonies attain during the first week a diameter of about 2 mm., and after 7 weeks a diameter of from 5 to 12 mm. If to such a petri-dish culture a small amount of water is added for a few minutes and afterward poured off, new colonies frequently arise after some days in other parts of the dish, in consequence of the removal of organisms from the old colonies. The successful hanging-drop cultures in the above-mentioned nutrient medium were composed of (1) the fungus, (2) the fungus and the red symbionts, and (3) the red symbionts.

The mycelium grows without much difficulty, the distance of the hyphae from each other being much greater than in the lichen. In this case no further description seems necessary. When the fungus was kept in contact with the one-celled organisms, I observed the same type of growth of the mycelium; some parts of this mycelium are not surrounded by the red cells, whereas other hyphae are in touch with their natural companions although they are never crowded. Sometimes the nutrient medium was somewhat saturated with the red pigment, and hyphae which died were sometimes slightly pink. The red organisms are often to be observed in the agar independently of the fungus, as though they were in pure culture; they can then be easily observed. They are of the same size as when found round the hyphae, never forming, as far as I was able to observe, any filaments. Sub-cultures could be made without difficulty.

When small pieces of the lichen were placed on a slide, moistened, and covered by a cover glass, they were able to develop further. In such a case the hyphae were much closer to each other than in the hanging-drop culture on agar. The red cells were also more crowded round the hyphae, although not as abundant as when in their natural state.

I was unable to bring both symbionts together when they were at first grown independently in pure culture. The fault, no doubt, lay in the methods employed.

When both symbionts of *Chiodecton sanguineum* derived from a dry situation are somewhat loosened and observed under a magnification of 1200 times, the red cells surrounding the hyphae are seen to be motionless; those which are detached, however, show an active motility which does not cease when they are killed in 95-percent alcohol or in any one of several other media. The movement, therefore, is Brownian. I was unable to stain flagella, and from the lack of evidence of active movement, it may be considered probable that no flagella are present.

When the lichen has been thoroughly moistened and kept in a petri dish or other container in order to prevent evaporation, there appears after 8 to 14 hours an innumerable amount of smaller red one-celled organisms, having



a length of from 0.5 to 1 micron and a thickness of from 0.3 to 0.5 micron. They too are motile, but do not suggest under any circumstances the actions of gametes and zoospores characteristic of many algae. Cells of all lengths from 0.5 micron to 2 microns are to be found.

Some of the smaller cells have also been observed round the hyphae, and in a few instances it was noticed that a small cell was connected with one slightly larger; which fact suggests that the small cells originate by ordinary division, and that they are never formed in large or small numbers *within* the larger red cells.

After the pigment had been extracted with pure methyl alcohol and the cell was afterward treated for staining, no nucleus appeared after staining with anilin blue, diluted Heidenhain's iron-alum haematoxylin, or with other stains, although for control several species of *Chlamydomonas*, *Pleurococcus*, *Zygnema*, *Ulothrix*, and a true *Trentepohlia* living against the trunk of a tree were so stained successfully. Meyer (7) states that he was able to stain easily the nucleus of *Trentepohlia umbrina* with haematoxylin, and Karsten (5) stained the nucleus in methyl green-acetic acid.

It is supposed that species of the alga *Trentepohlia* Mart. are associated with the fungus of *Chiodecton*. *Trentepohlia* belongs to the *Chroolepidaceae*. The life cycle as well as descriptions of species have been given by several authors. This alga forms thread-like colonies, each thread being composed of several cells, which, however, are never as small as the symbionts of *Chiodecton sanguineum*. Migula (8) describes various European species, some of which are shown on colored plates, the narrowest species *T. lagenifera* (Hild.) Wille having a width of 3 to 10 microns, sometimes to 15 microns. *T. abietina* (Flot.) Hansg. is 6 to 9 microns wide and its length is to three times the width. *T. (?) endophytica* (Reinsch) De Toni has a width and length of 4.5 to 6 microns. All other species are considerably larger. Most are to be found on stems of trees. Karsten (5), de Wildeman, and others (12, 13) who describe species from Java (the latter author also from other regions), describe clearly the general characteristics of *Trentepohlia*, but none of the descriptions or figures suggest the slightest resemblances with the symbionts observed in *Chiodecton sanguineum*.

Oltmanns (10) and Karsten (5) state that the cells of at least several species contain a pigment (haematochrom) which is present in numerous small drops. In *Trentepohlia umbrina* Bornet, which is brownish-red, the haematochrom does not dissolve in water or in dilute alcohol; it dissolves with difficulty in absolute alcohol, and better in ether or chloroform. When a cell is dead, the pigment becomes distributed uniformly throughout the cell. It becomes black in iodine and chlor-zinc-iodid, dark blue in sulfuric acid, and deep brown-black in osmic acid. The zoospores are flattish and attain a length of 11 microns; they possess two cilia, which are not easily visible and are longer than the spore and actively motile. They are red, the color being due to numerous drops of haematochrom. The nucleus is

made visible by methyl green-acetic acid. In other species the color of the pigment varies from yellow to orange red.

Oltmanns (10) summarizes quite extensively the work done on the Chroolepidaceae and treats very fully the genera *Cephaleuros* and *Trentepohlia*. The writer found on trunks of trees species of these two genera, which were used for comparison. Neither among other groups of green algae nor among the Cyanophyceae is there anything suggestive of the symbionts found by the writer in *Chiodecton*. It remains, therefore, to compare these organisms with the purple bacteria (*Rhodobacteriaceae*). This group is divided into *Thiorhodobacteriaceae* and *Athiorhodobacteriaceae* (6). The former are well known on account of the presence in their cells of sulfur compounds; they exert a strong oxidizing influence upon sulfuretted hydrogen. These sulfur bacteria are not uncommon in or near sulfur springs and in other places where sulfuretted hydrogen is available. The second group does not contain sulfur. It has been studied with much care by Molisch (9). To this group the symbionts of *Chiodecton sanguineum* apparently belong.

The *Rhodobacteriaceae* seem to form rather a physiological than a systematic group. There are genera which remind one of *Coccaceae*, of *Bacteriaceae*, and of *Spirillaceae*. They are in need, however, of more detailed investigation. They are all characterized by the possession of a red pigment, namely, bacteriopurpurin, which is not confined to certain bodies but is found throughout the entire cell. This is also evidently true of the organisms here described, unlike *Trentepohlia*, whose pigment is found in clear drops, which in some species or at certain periods may be visible only in certain parts of the cell. Molisch (9) has examined bacteriopurpurin spectroscopically, but his results are not the same with the different species he studied. He distinguishes bacteriopurpurin *A* obtained from *Rhodobacillus palustris*, which forms a carmine-colored solution in sulfur bicarbonate and a brownish carmine solution in chloroform; and bacteriopurpurin *B*, which is violet in sulfur bicarbonate and orange-red to brown-red in chloroform. He describes in detail the spectrum of bacteriopurpurin as well as other characteristics. Various species described by Molisch came from swamps, rivers, and the sea.

Engelmann (3) has shown that, although light works indifferently or harmfully to most bacteria, it is to many purple bacteria advantageous. The bacterial symbiont of *Chiodecton* lives partly directly exposed to the light, as far as the individuals are concerned which live about the margins of the lichen, and partly in relatively deep shade, namely, on the lower surface of the lichen. I have never had much success with pure cultures in the darkness; the few which succeeded were not so well developed as those of the same age growing in the light.

Winogradsky (14) was very uncertain of the significance of the bacteriopurpurin. Engelmann (3), however says:

Das Bakteriopurpurin ist ein echtes Chromophyll, insofern es in ihm absorbierte aktuelle Energie des Lichtes in potentielle, chemische Energie verwandelt.

Although Molisch was unable to observe that carbon dioxid was used and oxygen given off, nevertheless he finds that the organisms are rather dependent upon light. Molisch suggests that the purple bacteria are a link between the colorless bacteria, which use organic substances without the aid of light, and the green organisms which are dependent upon light. The purple bacteria approach the ordinary bacteria in their ability to assimilate organic substances in the darkness, and approach the green organisms so far that they have adapted themselves to the light and are able under the influence of light to consume organic substances more advantageously. They are, however, unable to assimilate CO<sub>2</sub>. From previous statements there are indications that purple bacteria may be able to live as symbionts and form a lichen, although this has not heretofore been observed. As in the case of many lichens, it is very difficult to state the reciprocal advantages of both symbionts.

There seems to be no doubt that the small red organisms here described belong to what are at present termed Rhodobacteriaceae.

Dr. Felix Löhnis, to whom I owe many kind suggestions, says:

Your tests which have shown that merely the Brownian movement was visible in water as well as in alcohol would suffice, I think, to answer the question concerning flagella. And the habitat I am sure leaves no doubt in regard to their relation to sulfur. Their distinctly bacterial form, together with their purple color, justifies fully, I believe, your inclination to class them as a Rhodobacterium species. The change from large to small forms and *vice versa* is common to all bacteria. Lankester observed this with his *Bacterium rubrum* about fifty years ago, and Winogradsky's rejection of these results is not well founded.

This heretofore unknown purple bacterium I propose to call *Rhodobacterium lichenophorum* nov. spec., with the following description:

Cells of a length of 0.5 to 2 microns and of a width of 0.3 to 0.5 micron. They are mostly rod-shaped but a few are slightly comma-shaped. The cell content of each individual is clearly filled with a deep red bakteriopurpurin. The organism possesses no flagella, its apparent motility being due to Brownian movement. It stains in carbol-fuchsin and in Löffler's methylene blue. No spore-formation has been observed. It develops when in contact with the fungus in the light as well as in shade. Pure cultures can be made on agar, but in this case do not develop well in darkness. The organism is found at least in symbiosis with *Chiodecton sanguineum* (Sw.) Waino.

As it is supposed that the lichen genus *Chiodecton* lives in symbiosis with *Trentepohlia*, an alga, instead of with a purple bacterium, I suggest that the name of the species of lichen in question should be changed to *Rhodobacteriophora sanguinea*. It would not be surprising if *Rhodobacterium lichenophorum* were found also to occur occasionally upon other species of lichens.

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# THE CHROMOSOME MORPHOLOGY OF VELTHEMIA, ALLIUM, AND CYRTANTHUS

WM. RANDOLPH TAYLOR

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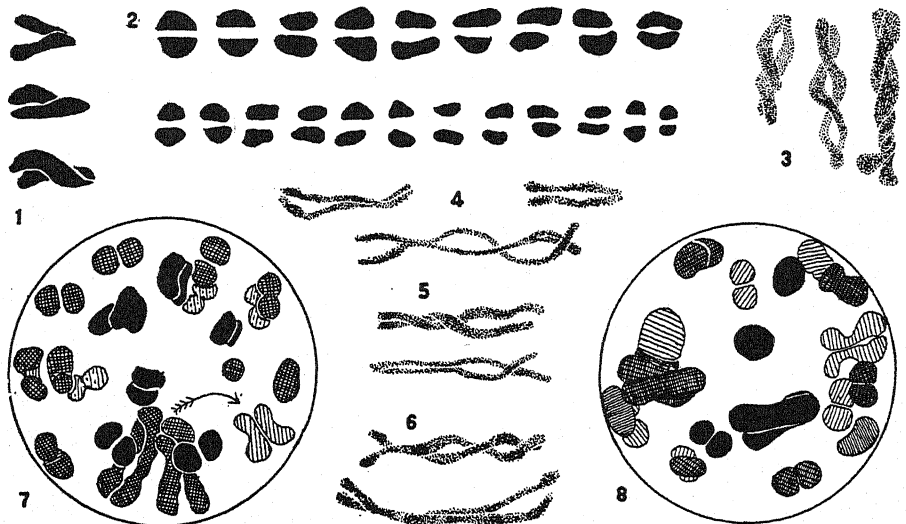
The specific identity of chromosomes has acquired new importance with the accumulation of evidence favoring the interpretation of the arrangement of genes as in effect linear. Where particular groups can be correlated with particular chromosomes, a criterion may be established whereby a cytological analysis of a plant may be used in genetical studies, either in the prediction or in the determination of the results of crosses. For the purpose of direct analysis, the matter of chromosome continuity between cell generations is exceeded in immediate importance by that of the individuality of form as a means of recognition. Until recently the only criterion of this sort has been the relative size of the chromosomes of a given complex, which is sometimes not sufficient to distinguish between the members of a group. Recent work by Sakamura and others, as reviewed by the writer (23), has indicated the presence of constrictions in plant chromosomes in positions so constant as to indicate that they are as stable a part of its morphology as any other feature. Since the preparation of this review, certain interesting reports have come to hand extending the record of satellites and constrictions as definite features. Delaunay (5) studied species of *Muscari*, figuring the differences between them. His satellites are not illustrated as always present on each homolog, but this may not be significant. M. Nawaschin (14) reports satellites also on *Crepis virens*. These papers have been unavailable to the writer except as reported by Tischler (25). Stomps (20) figures large satellites in *Narcissus*. These appear to be attached to the distal end of the chromosome, and are comparable to the elements in *Gasteria* rather than in *Galtonia* or *Crepis*. The present writer reviewed the conditions present in species of *Gasteria*, and discussed the forms under which the individuals of the complex appear in the stages of the maturation and vegetative mitoses, indicating that there is one type of constriction related to fiber-attachment and another which is independent. Neither appeared during the first maturation division, and the latter completely only in vegetative sporophyte mitoses, but the different chromosome types could be recognized in the different mitoses by other characters.

For the present paper the study of microsporogenesis was conducted entirely on slides prepared by the smear method, which has been described in detail elsewhere (24). The roots were sectioned in paraffin as usual. The fixing fluid used was that found most dependable for *Gasteria*, no. 9, composed of 2 percent osmic acid in 2 percent chromic acid, 1.5 cc.; 10

percent chromic acid, 0.2 cc.; 10 percent acetic acid, 2.0 cc.; distilled water, 8.3 cc.; maltose in varying amounts of about 1-2 percent. The best quantity of this last has not been determined, but it seems to prevent clumping of the chromatin elements and general shrinkage to a large degree. The *Allium* and *Cyrtanthus* material was fixed in this same fluid. All material was stained by the iron-alum-haematoxylin method, and *Allium* with the safranin-gentian violet-orange G combination also. Observations were made with a Spencer binocular microscope equipped with 1.40 n. a. achromatic condenser and Zeiss 1.40 n.a. 2 mm. apochromatic objective and compensation oculars, giving a highly critical image. Drawings were made at 3000 diameters (except *Allium* at 3600) and reduced to 2000 (*Allium*, 2400) in reproduction.

#### VELTHEMIA VIRIDIFOLIA JACQ.

In the present study on *Velthemia viridifolia* Jacq. (Liliaceae), the attempt was made to follow the maturation divisions in microspore-formation and the vegetative divisions in the root tip, classifying the elements

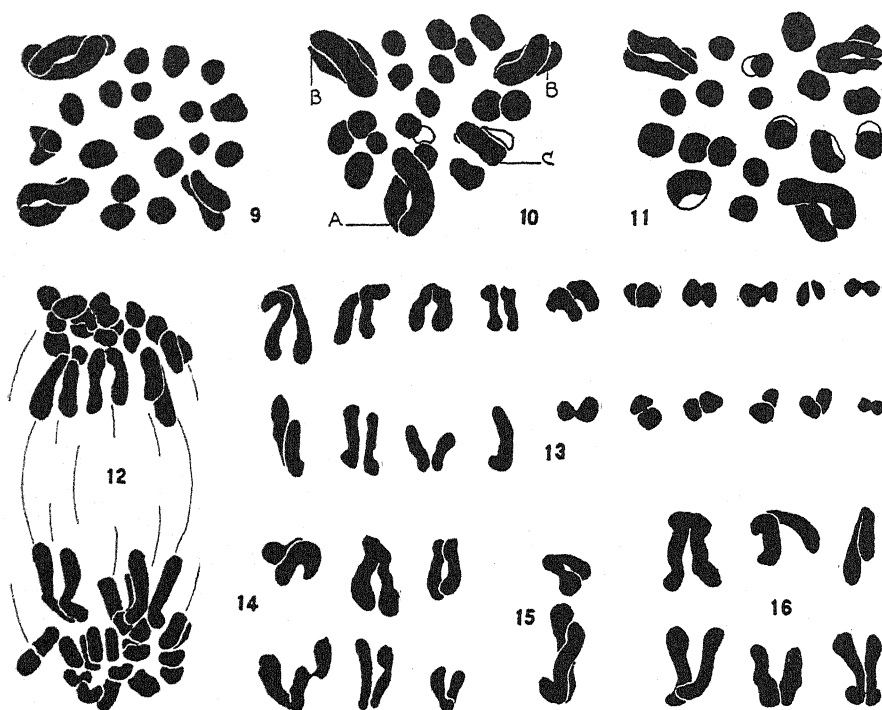


TEXT FIGS. 1-8. Diakinesis stages, microsporogenesis of *Velthemia viridifolia*.  $\times 2000$ .

by the available criteria at appropriate stages. Other vegetative tissues than the root tip, because of difficulty of fixation, are unsuitable, and the study on *Gasteria* indicated no fundamental differences between such.

The chromosome number in *Velthemia* sp.? was reported by C. Müller (13) as  $x = 10$ ,  $2x = 20$ . The material of *V. viridifolia* showed double this number. The compact form of most of the chromosomes largely compensated for the difficulties of observation introduced by this large number.

As the first maturation division prophase advances, a diakinesis stage is produced which is of striking appearance. In the earlier portion of it there lie in the nuclear cavity elements of two main classes. The larger of these are pale-stained, loosely twisted strands which appear to be three in number (text figs. 4-6, fig. 3 slightly older). With these are smaller elements which are much more sharply defined and are more deeply stained, although the figures over-emphasize this last feature. All of these smaller elements are not of the same size, and two classes at least are visible. One has elements much the larger and more or less twisted about each other (text fig. 1).



TEXT FIGS. 9-11. Metaphase plates, first maturation division, microsporogenesis of *Veltheimia viridifolia*. FIG. 12. Side view of anaphase, same species. FIGS. 13-16. Selected split chromosome pairs from four anaphase figures, same species.  $\times 2000$ .

The other has smaller elements which are more oval or hemispherical (text fig. 2). These vary much in size in a given cell, but since it was impossible to analyze completely individual cells with these bodies distributed around the periphery of the nucleus at various angles, no definite classification of them could be made. In text figures 1-6 only chromosome pairs lying with their longest axis nearly or quite at right angles to the line of vision are included. As diakinesis passes on toward the dissolution of the nuclear membrane, the chromosome elements condense somewhat further, but especially the larger and more diffuse elements (text figs. 7, 8). These can,

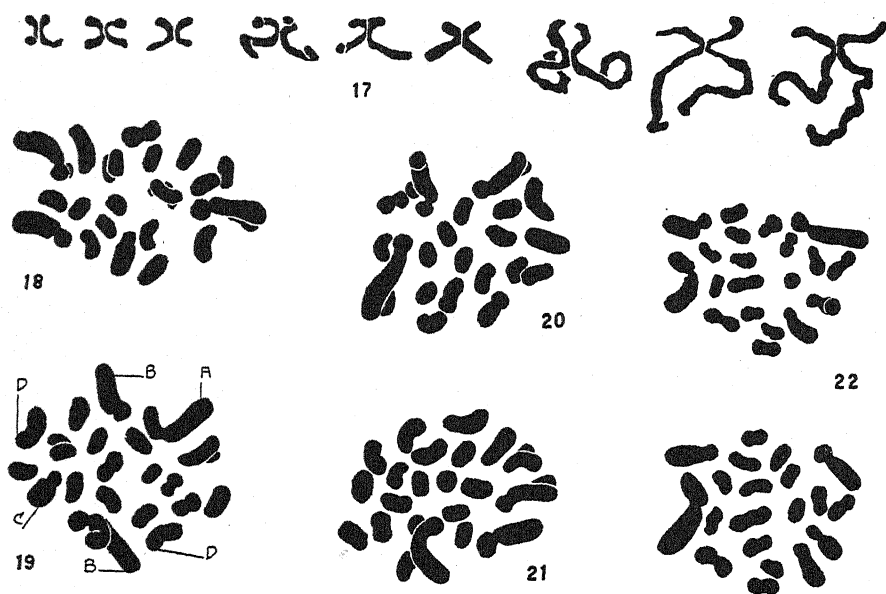
however, be recognized by their size right through the succeeding stages. Counts can usually be made at this time, but the number of chromosomes on the periphery which obscure each other renders this somewhat difficult.

On the metaphase plate of the first maturation division the chromosomes lie well separated (text figs. 9-11). Three elements are easily recognized, with a fourth somewhat less so. One, being markedly largest, can be designated *A*, the two somewhat smaller ones are nearly equal (*B*, *B*), and the fourth, less certain, is smallest (*C*). The rest of the chromosomes are smaller than these and round or oval, and attempts by a series of measurements to establish a classification of them gave no distinct results. The preparatory split for the second maturation division, which was so prominent in *Gasteria*, sometimes appears here at metaphase (text fig. 11, upper right), but is not fully effected until anaphase (text figs. 12-16). In *Velthemia* the smaller chromosomes lie toward the center of the anaphase group with the larger ones more marginal. The large number and close arrangement make the analysis of a complex at this stage difficult (text fig. 12). In text figure 13 is a partially complete set; the eight pairs of split chromosomes on the left represent the four split homologous pairs and are arranged in appropriate apposition. The twelve split chromosomes to the right are not arranged in pairs as homologues, but those in the upper row were proceeding to the upper pole and those in the lower row to the lower pole of the spindle. Among the larger pairs some had the spindle fiber attached quite a distance from the end (text fig. 13, upper left). This is indicated only by the flexure in the first maturation division, but is more distinct than it was in *Gasteria*. The left-hand homologues in text figure 14 show a phenomenon which occurred occasionally, namely, the abnormal separation of the homologues so that in this case part of one lobe of one of the upper elements is carried away on the corresponding lower one.

The chromosomes during interkinesis become more or less diffuse, but as the prophase spireme of the second maturation division condenses it becomes evident that here, as in *Gasteria*, the split chromosomes are coming out of the extended condition with the halves in a definite association. Whether the chromosomes were large or small and consequently represented by long or short strands, they always approximated at one definite point in a characteristic manner as shown in text figure 17. The division line between the elements was not as marked as in *Gasteria*, nor were the constrictions at the point of approximation as clear, but this is a matter of somewhat less favorable fixation. For that genus it was explained that this point of approximation corresponds to the point of spindle-fiber attachment for the ensuing division. In the metaphase of the second maturation division the chromosomes show more individual peculiarities than in the first (text figs. 18-21). The largest chromosomes are easily distinguished into one (*A*) with a large apical portion separated by a constriction from the main body, and two about the same size with a nearly spherical apical constricted



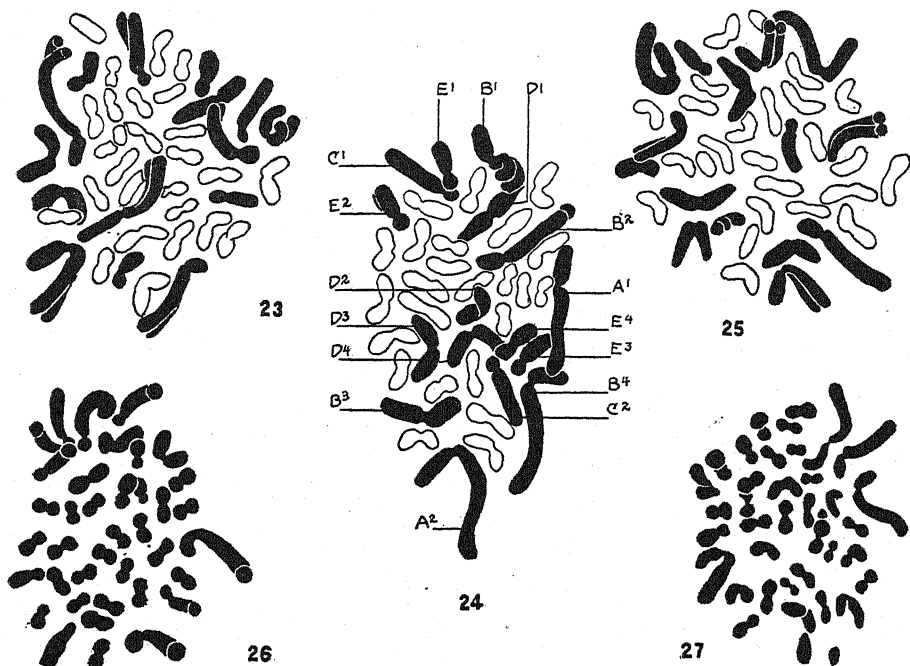
portion (*B*). Also there may be recognized chromosome *C* as a moderately short rod with a more or less distinct apical portion, and two chromosomes designated *D*, *D*, having a greater size and length than the other smaller elements and distinctly flexed. Probably elements of both the *C* and *D* groups are included in the class illustrated by text figure 1, but can not there be separated. In anaphase, of which text figure 22 shows the two groups from a single spindle, the constrictions become more pronounced, and it appears that part at least of the residue of small chromosomes are constricted near the middle. The anaphase stage, instead of affording because of this feature a favorable place for classifying the chromosomes, is poor because they lie with their most distinctive flexures or greatest length more or less nearly parallel to the axis of the spindle and it is only in oblique views that a correct idea of the form may be obtained, and in such views only a small part of any given set is in a favorable position.



TEXT FIG. 17. Prophase chromosomes, second maturation division, microsporogenesis of *Velthemia viridifolia*. FIGS. 18-21. Metaphase plates, same species. FIG. 22. Two anaphase groups from one cell, same species.  $\times 2000$ .

It will seem strange that the root-tip mitoses with their high number of 40 chromosomes should be indicated as the best ones for a complete morphological analysis of the complex in *Velthemia*, but a combination of circumstances favors them. While the disparity in size between the large and small elements is reduced, the large ones do not lose any of their distinguishing features and also they do not become as elongate and entangled as in *Allium*. The *A*, *B*, and *C* types are easily recognized when in favorable

positions, and other types are now also clear (text figs. 23-25). The type *D*, which was barely recognizable in the second maturation division, is here quite striking, being decidedly larger than any of the other chromosomes having a median fiber-attachment. There appears also a type (*E*) which is quite small and with the attachment constriction close to the end as in type *C*. There seem to be two pairs of these, or possibly only one. The uncertainty is caused by the form which the members of the residual group may show if slightly tilted on one end. This type was recognized in longitudinal views in anaphase. The remaining ten chromosomes all seem to have a median constriction and fiber-attachment. They appear to differ among themselves in size, but not sufficiently to classify them on such a



TEXT FIGS. 23-25. Metaphase plates, mitosis in root tip, *Veltheimia viridifolia*. FIGS. 26, 27. Anaphase groups, same species.  $\times 2000$ .

basis. In anaphase (text figs. 26, 27) the chromosomes show a somewhat reduced size and deeper constrictions. Their positions render this stage less favorable for study than the metaphase. In advanced stages the constricted region may lose the stain rather readily and appear notably paler as well as narrower than the bulk of the chromosome.

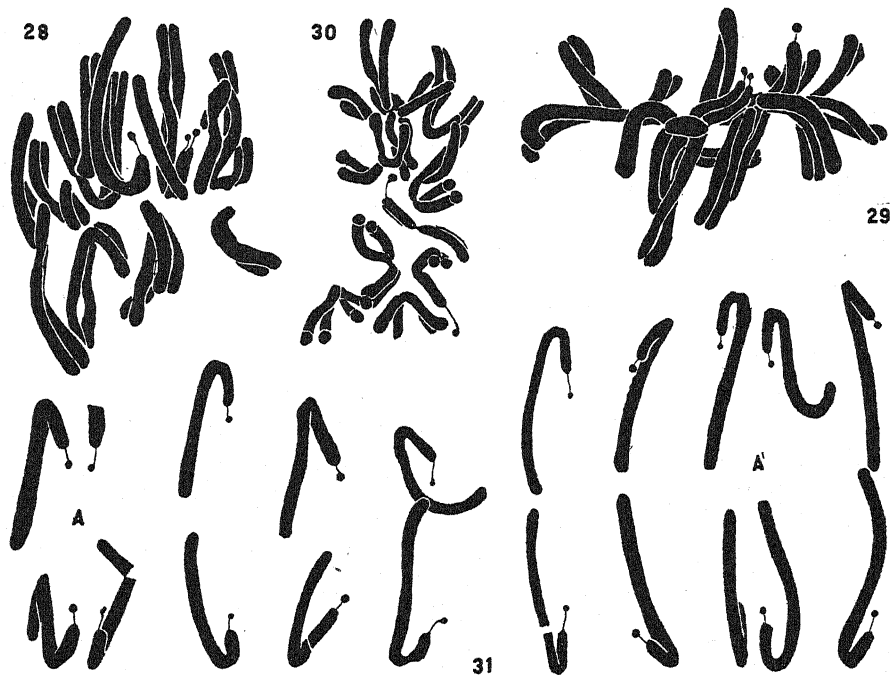
#### ALLIUM CEPA L.

Probably no other plant has received so much attention from cytologists and general students as the onion. A routine object for the study of

mitosis in root tips in almost every botanical laboratory, it seems incredible that any feature of its general chromosome configuration should have escaped recognition and record. The writer has gone through the available literature on the genus and has been surprised at the lack of agreement in the statements or which may be inferred from the figures regarding the shape of the chromosomes in relation to fiber-attachment. So far as mere root tip study is concerned the observational difficulties are great, but the studies of reduction divisions should have covered this point. Guignard (6) (*A. ursinum*) would indicate that the chromosomes in the second maturation division are attached at or near the middle point. Strasburger (21) (*A. odorum*) is mainly concerned with spindle-formation and his figures are not helpful for fiber-attachment. Ishikawa (7) (*A. fistulosum*) figures the maturation divisions with largely median attachment. Schaffner (18) (*A. cepa*) indicates by his drawings median attachment for some of the chromosomes. Němec (15) (*A. cepa*) in root-tip cells figures median, terminal, and subterminal attachments. Strasburger (22) (*A. fistulosum* and *A. fallax*) figures median and submedian attachments in maturation-division phases. Berghs (1) (*A. fistulosum*) does not cover this point. Merriman (10) does not figure or describe sufficiently analyzed stages that are appropriate. Miyake (11) (*A. victorialis*, *A. moly*, and *A. cepa*) figures median attachment in apparently all the chromosomes. Bonnevie (2) (*A. cepa*) figures some of the attachments as median, others as submedian, and later (3) gives one or two figures in which a pair of chromosomes seem to have a terminal attachment. Lundegårdh (8) (*A. cepa*) claims median segmentation (the fiber constriction). Dehorne (4) (*A. cepa*) shows median attachment. Lundegårdh (9) (*A. cepa*) shows in his figure 3g, which he cites as complete, both median and submedian constrictions. Mottier and Nothnagel (12) (*A. cernuum*) indicate that the attachment of the fibers is near or at the middle except for two chromosomes which appear as straight or hooked rods with the fiber-attachment at or near the end. V. Schustow (19) (*A. cepa*) gives some quite helpful figures, which indicate one pair as having the attachment fairly near the end, in the others median or in one pair submedian. Reed (17) (*A. cepa*) describes chromosomes with median attachment and V-shaped except one pair which has one of the long arms bent around toward the pole which the chromosome is approaching. He considers this shape as characteristic. Nothnagel (16) (*A. tricoccum*) figures median or submedian and terminal attachment.

The writer does not have the results of any complete analysis of the conditions in the onion to report at this time, but is able to add to the present descriptions the fact of the presence of satellites. In the cells of the root tip of *Allium cepa* one of the chromosome pairs has the fiber attached about one fourth the length from the end, and to the short arm is attached a very small satellite. This element was seen in both metaphase (text figs. 28-30) and anaphase (text fig. 31), in the latter being split and the parts

attached to each half. In figure 31 the pairs at *A* and *A'* are the homologues from the same spindle. There are two factors which may have served to prevent the previous recognition of this element. In the first place, it is attached to the short arm of the chromosome, which in anaphase is directed toward the spindle axis and is largely immersed in the apex of the sheaf of chromosomes, being visible only in exceptionally broad and loose figures. Secondly, it is small and easily destained. In unfavorable fixations it is either closely associated with the main part of the chromosome or entirely invisible and fused with it.



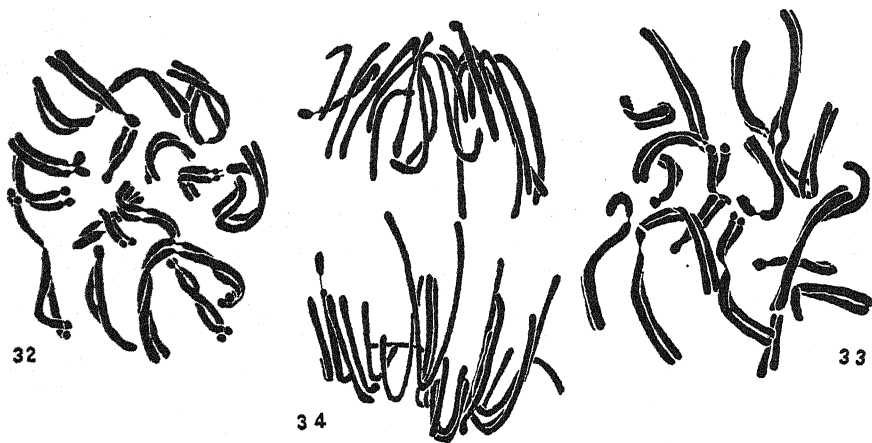
TEXT FIGS. 28, 29. Side view, metaphase plates, mitosis in root tip, *Allium cepa*. FIG. 30. Polar view, same species. FIG. 31. Anaphase chromosomes from several complexes, same species. At *A* and *A'* are the homologues from their respective figures.  $\times 2400$ .

#### CYRTANTHUS PARVIFLORUS Baker

In *Cyrtanthus parviflorus* Baker (Amaryllidaceae) there is presented a particularly interesting chromosome organization, but one which is, like that of *Allium* although in lesser degree, difficult to analyze because of the length and tangled character of the elements. There is no immediate prospect of maturation-division material becoming available, for the bulbs must almost invariably be sacrificed to get at the proper bud stages, and the supply of bulbs will not permit this. The root tips, on which the present report is based, were readily obtained from germinated seeds. When the observa-

tions on the somatic chromosomes in such a tangled complex are not verified by a study of the maturation divisions, a certain caution must be exercised in reporting the number of pairs of each type, but the types themselves are rather easily established.

Through the prophase stages the chromosomes are clearly split and when they reach the early metaphase-plate stage are still quite long (text figs. 32, 33). Later they tend to condense somewhat, and during anaphase become very slender indeed (text fig. 34). In the anaphase material available to the writer the constrictions were exceedingly marked, and a tendency was present for the chromosomes to destain at this point. There appear to be six types of chromosome present. The first and largest (text fig. 35, A) is represented by long split bands at metaphase with a marked constriction at the middle appearing as a diamond-shaped clear spot in the band. In anaphase these, separating, pass to the poles as V's with a median fiber-attachment. There are two pairs of this type of chromosome. The second type (text fig. 35, B) has a similar appearance except that the fiber-attach-



TEXT FIGS. 32, 33. Polar views, metaphase plates, mitosis in root tip, *Cyrtanthus parviflorus*. FIG. 34. Side view anaphase, same species.  $\times 2000$ .

ment is about one third the length from one end. This might look suspiciously like a damaged case of the previous type, but these were found in abundance under conditions precluding such a situation. One pair of this sort is present. The third type (text fig. 35, C) is particularly susceptible to fluctuations due to fixation. It is of medium size, with the fiber-attachment constriction about one third the length from one end, and shows under favorable conditions a notable constriction in the short arm, but if fixation is faulty, as usual when the observed cell is toward the center of the root, then the short arm is continuous. There is a single pair of these. The fourth type (text fig. 35, D) is very distinctive. Below the proximal end is the attachment constriction, and at the free distal end there is a large

portion separated by a marked constriction. It is comparable to the similar, but larger, chromosome in *Gasteria*, and as there is represented by but one pair. The remaining elements have but the proximal attachment constriction, and are of two sizes. The larger (*E*) seems to be present as two pairs and the smaller as one (*F*), but it is here that the maturation divisions are most needed to fix the number.



TEXT FIG. 35 A-F. Metaphase split chromosomes and anaphase chromosomes of the six classes in *Cyrtanthus parviflorus*.  $\times 2000$ .

#### SUMMARY

In *Veltheimia viridifolia* Jacq. there are 20 chromosomes in the haploid cell. In the diploid cell, where their characters are best observed, there is one pair with a short and a long arm, the former large, two pairs with a short and a long arm, the former small, one pair shorter and with the attachment closely subterminal, two pairs of about the same size and of median attachment, two pairs quite small with a closely sub-terminal attachment, and the others quite small and with median attachment.

In *Allium cepa* L. there is one pair of chromosomes which, instead of having median fiber-attachment and arms of equal length, has the fiber attached toward one end and this shorter arm bears a small satellite.

In *Cyrtanthus parviflorus* Baker there appear to be 16 chromosomes in the diploid cell, belonging to six classes. The largest two pairs have median attachment, the pair of the next size has an attachment about one third from one end. Another pair also has the attachment about one third distant from the one end, but the short arm is constricted. The remaining three classes have a closely sub-terminal fiber-attachment. Of these, one pair has a prominent element attached to the distal end. There appear to be two further pairs of medium size, and one pair quite small.

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# OBSERVATIONS ON THE POISONOUS PLANTS OF MICHIGAN

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This study is the result of a careful investigation of the flora of Michigan with a view to determine which of the poisonous plants listed in other states were found growing in this state. Michigan is not primarily a grazing state, so that the question of poisonous plants is not so vital as in some of the western states like Montana and Utah, but, nevertheless, there is evident a need for more information on the subject than is now available. I have included in this paper a list of the poisonous plants of the state, accompanied by a few notes on their distribution and poisonous nature where such information is available.

Not having included algae and fungi in the list of species, it might be well to mention the more important ones in this introductory note. Among the fungi the genus *Amanita* is by far the most poisonous of any of the umbrella type of mushrooms and should be avoided by people when collecting edible fungi. The spores of *Aspergillus* when inhaled produce a general mycosis in the lungs of animals. Ergot, the sclerotium stage of *Claviceps purpurea*, produces a poisonous effect on animals; in severe cases causing a loss of the extremities of the limbs, tail, and ears. Numerous cases of suspected poisoning from moldy silage have come to the author's attention, but no exact information is available as to the particular fungus causing the poisoned condition.

The algae as a group contain few poisonous species. Although algae may give water an objectionable appearance or odor, they rarely render it poisonous either to stock or to human beings. Furthermore, it is not at all probable that even decaying algae injure the water to the extent of making it poisonous. However, on account of the objectionable odor that the decomposing algae impart to water, and on account of their tendency to clog drain pipes, it is often highly desirable to get rid of them. Many algae are quite easily prevented from growing in water by treatment with copper sulfate at the rate of one part of sulfate to one million parts of water. The small quantities of this salt that go into solution are harmful to the algae but do not injure livestock or human beings.

## LIST OF SPECIES

### Polypodiaceae (Fern Family)

1. *Adiantum pedatum* L. Maidenhair Fern. Common in rich moist woods. Reported poisonous. Prussic acid present.

2. *Dryopteris* spp. Shield Fern. Occasional. Rootstock used for the expulsion of tape worm.
3. *Pteridium aquilinum* (L.) Kuhn. Brake. Common. Thickets and hillsides. Rootstock poisonous to cattle and horses. Prussic acid present.

#### Equisetaceae (Horsetail Family)

4. *Equisetum arvense* L. Field Horsetail. Common in damp grass-lands and moist road embankments. Dried plant poisonous. Horses most susceptible, sheep slightly.

#### Pinaceae (Pine Family)

5. *Juniperus communis* L. Juniper. Occasional on dry hillsides. Oil from fruit poisonous to people, leaves poisonous to goats.
6. *J. virginiana* L. Red Cedar. Occasional in dry, open places. Oil from berries produces abortion in animals.

#### Taxaceae (Yew Family)

7. *Taxus canadensis* Marsh. American Yew. Ground Hemlock. Occasional in woods. Fruit reported poisonous to some people, although children have been observed to eat freely of the berries.

#### Typhaceae (Cat-tail Family)

8. *Typha latifolia* L. Broad-leaved Cat-tail. Common in swamps. The stem and rhizome are ground up for food, which in some instances has proved poisonous to people.

#### Scheuchzeriaceae (Arrow-grass Family)

9. *Triglochin maritima* L. Seaside Arrow-grass. Shores of the Great Lakes and along borders of deer licks in the interior. When dry and eaten in large amounts, fatal to animals.

#### Gramineae (Grass Family)

10. *Hordeum jubatum* L. Squirrel-tail Grass. Occasional in sandy soil. Awns from heads break into segments and insinuate themselves about the teeth of animals, causing inflammation of the gums and falling of teeth. Other species of *Hordeum* such as the cultivated barleys produce indigestible balls in stomachs of cattle and horses.
11. *Bromus tectorum* L. Downy Brome-grass. Frequent in fields and waste places. Produces injuries similar to those produced by *Hordeum jubatum*.
12. *Stipa avenacea* L. Black Oat Grass. *S. spartea* Trin. Porcupine Grass. Dry woods in southern part of Lower Peninsula. Sharp-

pointed callus of fruit enters skin of sheep and other animals having long-haired parts. May produce internal injury when ingested.

13. *Cenchrus carolinianus* Walt. Sand-bur. Bur-grass. Hedgehog-grass. Occasional in sandy soil of southern part of the Lower Peninsula. Spine-covered fruit may work into the flesh of animals.
14. Millets (species of the genera *Chaetochloa*, *Panicum*, and *Echinochloa*). Common. Injurious nature same as that of cultivated barley. The Foxtail Millet (*Chaetochloa italica* (L.) Scrib.) is also poisonous to the kidneys of horses.
15. *Lolium temulentum* L. Darnel. Occasional in waste places and cultivated grounds. Poisoning due to fungus harbored in the seed.

#### Araceae (Arum Family)

16. *Arisaema triphyllum* (L.) Torr. Jack-in-the-pulpit. Indian Turnip. Corm poisonous to people when ingested and also may produce irritation of the skin.

#### Melanthaceae (Bunch-flower Family)

17. *Anticlea elegans* (Pursh) Rydb. Swamp Camas. Occasional in moist soils of Lower Peninsula. All parts of the plant are poisonous to horses, cattle, and sheep. By some authors the eastern form is considered a distinct species, *A. chloranthus*. All records of poisoning are based on the western form, the true *A. elegans*.
18. *Colchicum autumnale* L. Colchis. Meadow Saffron. Occasionally grown in gardens. Produces a poisonous honey. Bees which visit the blossoms often become intoxicated and in some instances are unable to return to the hives.

#### Convallariaceae (Lily-of-the-valley Family)

19. *Convallaria majalis* L. Lily-of-the-valley. Cultivated in gardens and escaped in places. All parts of the plant poisonous to man and animals. Toxic honey is obtained from the flowers.

#### Liliaceae (Lily Family)

20. *Allium canadense* L. Meadow Garlic. Common in moist meadows and thickets. Poisonous to cattle because of the irritating effect of the oil which is characteristic of the genus *Allium*.

#### Trilliaceae (Wake-robin Family)

21. *Trillium erectum* L. Ill-scented Wake Robin. Occasional in woods. Rootstocks poisonous, acting as an emetic.
22. *T. grandiflorum* (Michx.) Salisb. Large-flowered Wake Robin.

Common in woods. Poisonous properties same as those of *T. erectum*.

#### Smilacaceae (Smilax Family)

23. *Smilax herbacea* L. Carrion Flower. Common all over the state. Flowers poisonous.

#### Iridaceae (Iris Family)

24. *Iris versicolor* L. Large Blue Flag. Common in low grounds. Rootstock poisonous, causing congestion of gastro-intestinal tract, liver, and pancreas.

#### Orchidaceae (Orchid Family)

25. *Cypripedium* spp. Ladies'-slipper. Several species in the state. Found in moist woods and swamps. Produces a dermatitis resembling that produced by *Toxicodendron toxicodendron*.

#### Fagaceae (Beech Family)

26. *Fagus grandifolia* Ehrh. American Beech. Common in rich soil of Lower Peninsula. Fruit reported to produce enteritis.
27. *Castanea dentata* (Marsh) Borkh. American Chestnut. Occasional in rich soil of southeastern part of the state. Burs produce mechanical injuries.
28. *Quercus rubra* L. Red Oak. Common in all parts of the state. Acorns injurious to cattle. Leaves of all species of *Quercus*, when forming the sole food of animals, are poisonous.

#### Cannabinaceae (Hemp Family)

29. *Humulus lupulus* L. Common Hop. Frequent along banks of streams northward. Produces an inflammation of the pickers' hands.
30. *Cannabis sativa* L. Hemp. Frequent in waste places. Resin of plant produces narcotic effect.

#### Urticaceae (Nettle Family)

31. *Urtica dioica* L. Great Nettle. Occasional in waste places. Contents of stiff hairs produce intense inflammation in people and animals.
32. *U. gracilis* Ait. Slender Nettle. Common in dry soil. Poisonous properties same as those of *U. dioica*.
33. *Urticastrum divaricatum* (L.) Kuntze. Wood Nettle. Common in rich woods of central and southern Michigan. Poisonous nature same as that of *Urtica dioica*.

**Aristolochiaceae (Birthwort Family)**

34. *Asarum canadense* L. Wild Ginger. Common in rich woods all over the state. All parts of the plant produce a burning sensation.
35. *Aristolochia sipho* L'Her. Dutchman's Pipe. Cultivated. Flowers poisonous.

**Polygonaceae (Buckwheat Family)**

36. *Rumex acetosella* L. Sheep Sorrel. Common in dry fields and on hillsides. Reported poisonous to sheep and horses.
37. *R. crispus* L. Narrow Dock. Common in fields and waste places. Acts as an astringent.
38. *Fagopyrum fagopyrum* (L.) Karst. Buckwheat. Persistent in fields. Buckwheat cakes sometimes produce a dermatitis in people and hogs.
39. *Persicaria punctata* (Ell.) Small. Dotted or Water Smartweed. Frequent in swamps and wet places.
40. *P. hydropiper* (L.) Opiz. Water Pepper. Smartweed. Common in moist waste places all over the state. Both of the species of *Persicaria* here mentioned are looked upon with suspicion.

**Amaranthaceae (Pigweed Family)**

41. *Amaranthus spinosus* L. Spiny Amaranth. Rare in waste and cultivated soil. Produces mechanical injuries and bloat in animals.

**Chenopodiaceae (Goosefoot Family)**

42. *Chenopodium ambrosioides* L. Mexican Tea or Wormseed. Occasional in waste places. Oil from seed poisonous.
43. *Beta vulgaris* L. Sugar Beet. Cultivated in Lower Peninsula. Leaves when used for fodder sometimes cause bloat in animals. The roots when eaten by animals often cause renal calculi consisting of uric and phosphoric acid with lime.
44. *Salsola pestifer* A. Nelson. Russian Thistle. General in cultivated fields and waste places over entire state. Poisonous nature due to sharp spines which break under the skin and cause festering sores.

**Phytolaccaceae (Pokeweed Family)**

45. *Phytolacca americana* L. Poke or Pigeon-berry. Frequent in central and southern Michigan. Roots and seeds act as a slow but violent emetic, and also affect the nerves and muscles. Roots often mistaken for those of horse radish.

**Alsineaceae (Chickweed Family)**

46. *Alsine media* L. Common Chickweed. Abundant all over the state. Seeds when eaten in large quantities by lambs produce digestive disorders.

**Caryophyllaceae (Pink Family)**

47. *Agrostemma githago* L. Corn Cockle. Common in wheat fields. Wheat screenings which contain a large amount of cockle are poisonous to stock.

**Ranunculaceae (Crowfoot Family)**

48. *Caltha palustris* L. Marsh Marigold. Common in swamps. When fed with hay, produces in animals diarrhoea and stoppage of flow of milk.
49. *Actaea rubra* (Ait.) Willd. Red Baneberry.
50. *A. alba* (L.) Mill. White Baneberry. Both this and *A. rubra* are frequent in woods throughout the state and produce poisonous berries.
51. *Anemone quinquefolia* L. Wind Flower. Common in low woods all through state. All parts of plant act as an irritant.
52. *Clematis virginiana* L. Virgin's Bower. Common in low woodlands and along water courses. Juice a strong irritant, causing blisters or even ulcers.
53. *Ranunculus acris* L. Tall Buttercup. Frequent in fields and meadows. Sometimes causes death in animals resembling that from apoplexy.
54. *Thalictrum dioicum* L. Early Meadow Rue. Common in woods over entire state. Poisonous to stock.

**Berberidaceae (Barberry Family)**

55. *Berberis vulgaris* L. Common Barberry. Common over entire state. Berries used for jelly, which in some instances is said to be poisonous.
56. *Caulophyllum thalictroides* (L.) Michx. Blue Cohosh. Common in woods all over the state. Reported poisonous.
57. *Podophyllum peltatum* L. May Apple. Common in low woods in central and southern part of state. Roots and leaves drastic. Leaves when eaten by cows produce injurious milk. Roots are irritating to the eye, nose, mouth, and skin of people.

**Menispermaceae (Moonseed Family)**

58. *Menispermum canadense* L. Canada Moonseed. In woods along streams. Berries poisonous and often eaten by mistake for grapes.

**Papaveraceae (Poppy Family)**

59. *Papaver somniferum* L. Common Poppy. Occasional in waste grounds. Escaped from cultivation. From unripe capsule opium is obtained, which exerts a powerful sedative effect on the nervous systems of people.

60. *Sanguinaria canadensis* L. Bloodroot. Common in rich woods throughout the state. Poisonous nature due to sanguinarin which is a strong irritant.
61. *Chelidonium majus* L. Celandine. Occasional. Produces congestion of the lungs and liver and is also an excessive irritant.
62. *Bicuculla canadensis* (Goldie) Millsp. Squirrel Corn. Frequent in rich woods of Lower Peninsula. All parts of the plant contain the alkaloid cucullarin, which is poisonous to cattle when eaten in large amounts.
63. *B. cucullaria* (L.) Millsp. Dutchman's Breeches. Habitat and poisonous nature same as those of *B. canadensis*.

#### Cruciferae (Mustard Family)

64. *Sinapis alba* L. White Mustard. Occasional in waste places over entire state. Bad weed in fields of small grains. The seed, when reduced to a powder and made into a paste with cold water, acts as a powerful stimulant. The oil derived from this plant is a virulent, irritant poison.
65. *Armoracia armoracia* (L.) Britton. Common Horse Radish. Occasional in moist ground. Escaped from cultivation. Fleshy roots when ground used as a condiment by people. But when used in excess it becomes a powerful irritant of the urinary organs. In animals the poison produces violent colic.
66. *Brassica rapa* L. Common Turnip. Cultivated over most of the state. Seeds reported poisonous to cattle, causing a decrease in flow of milk, and abortion.
67. *Bursa bursa-pastoris* (L.) Britton. Shepherd's Purse. Common in fields and waste places all over the state. Reported poisonous.

#### Crassulaceae (Orpine Family)

68. *Sedum acre* L. Mossy Stonecrop. Occasional on rocks and along roadsides. Juice of plant irritating to the skin.

#### Rosaceae (Rose Family)

69. *Rubus* spp. Widely distributed over the state. No species of raspberries or blackberries is known to be poisonous, but the bristles and spines on various species frequently inflict injuries which may become inflamed and develop pus.
70. *Fragaria* spp. Strawberry. The wild species *F. americana*, *F. canadense*, and *F. virginiana* are known in the state. *F. chiloensis* is cultivated extensively. Poisoning sometimes results from eating the fruit.
71. *Rosa* spp. Rose. The numerous species in the state possess spines

which inflict injuries upon cattle and people. They are especially troublesome in grain fields.

### Malaceae (Apple Family)

72. *Malus* spp. Apple. The various species are quite generally cultivated. All species contain a small amount of the glucoside amygdalin, which is converted by fermentation into hydrocyanic or prussic acid. Wilting leaves are poisonous to stock because of the prussic acid present.

### Amygdalaceae (Peach Family)

73. *Padus nana* (DuRoi) Roemer. Choke Cherry. Common all over the state along river banks and in rocky situations.
74. *P. virginiana* (L.) Mill. Wild Black Cherry. Frequent in woods and open places in central and southern part of Lower Peninsula. Also found in the Upper Peninsula. Of several species in the state both native and cultivated, the above-named species are the most poisonous. Cattle are often poisoned by eating wilted leaves which contain prussic acid.

### Caesalpinaceae (Senna Family)

75. *Gymnocladus dioica* (L.) Koch. Kentucky Coffee Tree. Occasional in rich woods. Central and southern. Fruit poisonous to people.

### Fabaceae (Pea Family)

76. *Baptisia leucantha* T. & G. Large White Wild Indigo. Rich soil. Rare in central and southern Michigan.
77. *B. tinctoria* (L.) R. Br. Wild Indigo. Occasional in dry soil in southern part of state. This species and *B. leucantha* act as emetics and cathartics.
78. *Crotalaria sagittalis* L. Rattle Box. Occasional in dry, open places. Poisonous to horses, producing a general bodily decline in vigor. In the west the poisoned condition is known as "Missouri bottom" disease.
79. *Lupinus perennis* L. Wild Lupine. Central and southern part of state in dry, sandy soil. Poisonous when mature to horses and sheep. Most noticeable symptom of poisoning is a great frenzy; death is accompanied by marked convulsions.
80. *Trifolium hybridum* L. Alsike Clover. Occasional.
81. *T. pratense* L. Red Clover. Common over the entire state. This species and *T. hybridum* occasionally produce bloat in animals.
82. *T. incarnatum* L. Crimson Clover. Occasionally cultivated. Hairs from mature plants form indigestible masses in the stomachs of horses.



83. *T. repens* L. White Clover. Commonly cultivated over the entire state. Produces bloat in cattle and slobbering in horses.
84. *Melilotus alba* Desv. White Sweet-clover. Occasional along roadsides and waste places. Sometimes grown as a fodder plant, but sometimes reported as poisonous because of the coumarin contained in the plant.
85. *Medicago sativa* L. Alfalfa. Cultivated. When fed green in large quantities produces bloat.
86. *Robinia pseudo-acacia* L. Locust Tree. Occasional. Bark poisonous. Honey from flowers poisonous.
87. *R. viscosa* Vent. Clammy or Honey Locust. Occasional. Barbs and leaves contain a powerful poison which has proved fatal to people. Children have been poisoned by eating the roots. Seeds also poisonous.
88. *Vicia sativa* L. Common Vetch. Occasional in fields and waste places. Sometimes causes bloat in horses.
89. *Astragalus mollissimus* Torr. Woolly Loco-weed or Crazy-weed. Occasional in southern Michigan. In the west this species is reported as causing a poisoned condition of the nervous system, the animals affected having unnatural movements and frenzied actions.
90. *Lathyrus* spp. Flat Pea. Several species in state. Seeds poisonous to horse and pig, causing debility of the rear parts and paralysis of the larynx.
91. *Phaseolus multiflorus* Willd. Scarlet Runner Bean. Cultivated as ornamental vine. Ripe seeds poisonous.
92. *P. lunatus* L. Lima Bean. Cultivated. Wilted leaves poisonous to stock because of prussic acid present.
93. *Glycine hispida* Maxim. Soy Bean. Cultivated for forage. Harmful to cattle when fed in large amounts because of purgative properties.
94. *Wistaria chinensis* DC. Chinese Wistaria. Woody twiner. Cultivated. Flowers poisonous.

#### Geraniaceae (Geranium Family)

95. *Erodium cicutarium* (L.) L'Her. Stork's Bill. Occasional in waste places and fields in Lower Peninsula. Sharp-pointed outgrowths from dry, hard fruit bury themselves in the flesh and inflict injuries upon animals.

#### Oxalidaceae (Wood-sorrel Family)

96. *Xanthoxalis stricta* (L.) Small. Yellow Wood-sorrel. Common throughout the state in woods and fields. Leaves when eaten in considerable quantities cause convulsions in people.

**Linaceae (Flax Family)**

97. *Linum usitatissimum* L. Common Flax. Cultivated and escaped. When the seeds are ground into a meal and this is fed in concentrated form, digestive troubles result in hogs and cattle because of the prussic acid present.

**Simarubaceae (Tree-of-heaven Family)**

98. *Ailanthus glandulosa* Desf. Tree-of-heaven. Occasional. Bark and leaves poisonous. Flowers produce irritation of skin in people.

**Euphorbiaceae (Spurge Family)**

99. *Chamaesyce* spp. and *Tithymalus* spp. Both genera commonly known as Spurge. Several species of each in the state in sandy soil. All are more or less irritating and in drying give off disagreeable odors.
100. *Ricinus communis* L. Castor Oil Plant. Cultivated as an ornamental plant. Seeds produce well known castor oil which is a mild and safe purgative. The seed pulp contains the acrid substance ricin. Three seeds have caused death in man, and they are ten times more purgative than the oil. Seeds or oil cake also poisonous to horses, cattle, and poultry. Ricin when injected into the circulation is more poisonous than strychnin, prussic acid, or arsenic.

**Anacardiaceae (Sumac Family)**

101. *Toxicodendron toxicodendron* L. Poison Ivy. Common all over the state in dry situations. Contact with the plant produces an intense inflammation of the skin of some people.
102. *T. vernix* (L.) Kuntze. Poison Sumac or Poison Elder. Common in swamps. Poisonous nature same as that of *T. toxicodendron*.

**Celastraceae (Staff-tree Family)**

103. *Celastrus scandens* L. Climbing Bittersweet. Frequent in rich soil throughout the state. Leaves poisonous to horses.
104. *Euonymus atropurpureus* Jacq. Wahoo or Burning Bush. Occasional in central and southern part of state. Fruit poisonous.

**Aesculaceae (Buckeye Family)**

105. *Aesculus hippocastanum* L. Horse Chestnut. Occasionally cultivated. Seed and leaves poisonous.

**Rhamnaceae (Buckthorn Family)**

106. *Rhamnus alnifolia* L'Her. Buckthorn. Common in swamps in all parts of the state. Fruit poisonous.

**Hypericaceae (St. John's-wort Family)**

107. *Hypericum* spp. St. John's-wort. Several species common in state. Poisonous to sheep, cattle, and horses when eaten, because of a substance which is readily absorbed from the alimentary tract and deposited in the unpigmented skin, where it acts as a sensitizing agent on the tissue. The sensitized skin reacts upon exposure to sunlight, and there is set up an intense itching which the animal attempts to reduce by vigorous scratching or rubbing.

**Violaceae (Violet Family)**

108. *Viola tricolor* L. Hearts' Ease or Pansy. Cultivated. Poisonous nature due to an acrid, bitter principle which acts as an emetic. The most characteristic symptom of its action is an offensive odor of the urine, like that of the cat. On the skin it causes burning, stinging, and itching followed by encrusted eruptions.

**Cactaceae (Cactus Family)**

109. *Opuntia humifosa* Raf. Western Prickly Pear. Occasional in dry, sandy, or rocky soil. Barbed hairs at bases of spines cause indigestible masses in stomachs of animals. Fruit produces diarrhoea when eaten.

**Thymeleaceae (Mezereum Family)**

110. *Dirca palustris* L. Leather-wood. Frequent in wet woods over entire state. Fresh bark when applied to skin of people produces redness and vesication, the sores being difficult to heal. Fruit also poisonous.

**Araliaceae (Ginseng Family)**

111. *Echinopanax horridum* (J. E. Smith) Decne. & Planch. Hercules' or Devil's Club. Found only on rocky soil of Isle Royale, Lake Superior, so far as known. Prickly spines are quite irritating.

**Ammiaceae (Carrot Family)**

112. *Conium maculatum* L. Poison Hemlock. Occasional in waste places in central and southern Michigan. Poisonous to cattle and people. Cattle show loss of appetite, salivation, bloating, loss of muscular power, and rapid, feeble pulse. In people the power of sight is often lost, but the mind remains clear until death from the gradual paralysis of the lungs. Seeds occasionally accidentally eaten for that of anise, the leaves for parsley, and the roots for parsnips. Poisoning also sometimes occurs from blowing whistles made from the hollow stems.
113. *Cicuta maculata* L. Spotted Cowbane or Water Hemlock. General in swamps and low grounds over the entire state, but particularly

- abundant in Saginaw County where serious losses of stock have occurred. Poisoning similar to that of poison hemlock except for the appearance of convulsions in addition to the symptoms noted above. People occasionally mistake the roots for those of parsnip.
114. *Pastinaca sativa* L. Wild Parsnip. Occasional in waste places. Poisonous to people, producing a very severe dermatitis.
115. *Daucus carota* L. Wild Carrot. A far too common weed in many parts of the state. Leaves when wet with dew cause vesication.
116. *Heracleum lanatum* L. Cow Parsnip. Common throughout the state in moist ground. Leaves produce irritation.

#### Ericaceae (Heath Family)

117. *Kalmia angustifolia* L. Sheep Laurel or Lambkill. Occasional in moist soil. Leaves poisonous to sheep and cattle.

#### Oleaceae (Olive Family)

118. *Ligustrum vulgare* L. Privet. Used in hedge rows. Leaves and fruit poisonous.

#### Asclepiadaceae (Milkweed Family)

119. *Asclepias syriaca* L. Common Milkweed. Common in fields and waste places throughout the state. Poisonous to stock.
120. *A. verticillata* L. Whorled Milkweed. Occasional in barren regions of southern part of Lower Peninsula. Poisonous to sheep.

#### Convolvulaceae (Morning-glory Family)

121. *Convolvulus* spp. Bindweed. Several species in the state. Roots poisonous to hogs because of the purgative action.
122. *Ipomea purpurea* (L.) Lam. Morning-glory. Cultivated. Poisonous nature same as that of genus *Convolvulus*.

#### Cuscutaceae (Dodder Family)

123. *Cuscuta* spp. Dodder. Several species in the state. Cause digestive troubles in horses and cattle.

#### Labiatae (Mint Family)

124. *Leonorus cardiaca* L. Motherwort. Common in waste places. Produces mechanical injuries and dermatitis.
125. *Glechoma hederacea* L. Ground Ivy. Occasional as an escape. Said to be poisonous to horses.

#### Boraginaceae (Borage Family)

126. *Borago officinalis* L. Borage. Occasional in waste places. Bristles produce irritation.

**Solanaceae (Potato Family)**

127. *Solanum dulcamara* L. Bittersweet. Nightshade. Common in waste places or moist thickets in central and southern Michigan. Fruit poisonous.
128. *S. tuberosum* L. Irish Potato. Cultivated all over the state. Wilted green stems and leaves poisonous. "Sunburned" tubers poisonous.
129. *S. rostratum* Dunal. Beaked Nightshade or Buffalo Bur. Occasional. Stiff yellowish prickles cause mechanical injury to animals.
130. *S. nigrum* L. Black Nightshade. Common in waste places in central and southern Michigan. All parts of the plant poisonous to animals, producing stupefaction, staggering, loss of consciousness, and convulsions. Death due to paralysis of lungs. Berries harmless if fully ripe, poisonous when green.
131. *Hyoscyamus niger* L. Black Henbane. Rare in waste places. Poisonous to animals.
132. *Nicotiana tabacum* L. Tobacco. Cultivated occasionally in southern Michigan. Leaves poisonous, because of the presence of nicotin.
133. *Capsicum annuum* L. Red Pepper. Cultivated. Fruit when used too long as a stimulating plaster by people may cause vesication. When eaten by animals it causes gastro-enteritis.
134. *Datura stramonium* L. Common Jimson Weed or Thorn-apple. Frequent escape from gardens in central and southern Michigan. Leaves and seeds poisonous, causing dilation of eye. Honey from flowers poisonous. Seeds may cause temporary insanity or even death.

**Scrophulariaceae (Figwort Family)**

135. *Linaria linaria* (L.) Karst. Butter and Eggs. Common in fields and waste places. Suspected of being poisonous.
136. *Digitalis purpurea* L. Purple Foxglove. Cultivated. Gastro-intestinal irritant in people and animals. Seeds especially poisonous.

**Rubiaceae (Madder Family)**

137. *Cephalanthus occidentalis* L. Button-bush. Common in swamps of Lower Peninsula. Leaves poisonous, due to a bitter glucoside.

**Caprifoliaceae (Honeysuckle Family)**

138. *Sambucus canadensis* L. Common Elder. Common in moist soil throughout the state. Leaves, flowers, and roots poisonous.
139. *S. racemosa* L. Red-berried Elder. Common in the northern half of the Lower Peninsula and somewhat common in the southern half. Berries suspected of being poisonous.

**Lobeliaceae (Lobelia Family)**

140. *Lobelia inflata* L. Indian Tobacco. Occasional in fields and thickets. Poisonous substance contained in the plant produces nausea, prostrations, and convulsions.

**Cichoriaceae (Chicory Family)**

141. *Cichorium intybus* L. Chicory. Frequent along roadsides and in fields throughout the state. When fed to cattle in large quantities it imparts a bitter flavor to milk and butter.
142. *Sonchus* spp. Sow Thistle. Several species in the state. They produce mechanical injuries to stock and also possess an active bitter principle.

**Ambrosiaceae (Ragweed Family)**

143. *Iva xanthiifolia* Nutt. Burweed or Marsh Elder. Occasional in moist soil or waste places. This plant produces a large amount of pollen which is looked upon as in part responsible for hay fever among people. The pollen is an irritant of the nasal mucous membrane.
144. *Ambrosia* spp. Ragweed. Several species well scattered over the state. Pollen poisonous as in *Iva xanthiifolia*.
145. *Xanthium commune* Britton. Cocklebur. Common in moist grounds in all parts of the state. The prickly fruit is indigestible, and the barbs sometimes produce injury to the animals feeding upon it. The hairs of the plant cause itching.

**Compositae (Thistle Family)**

146. *Eupatorium urticaefolium* Reichard. White Snake-root. Reported for a few locations in rich woods of the state. Produces a disease known as "trembles" in cattle, horses, and sheep, and "milk sickness" in people.
147. *Solidago* spp. Golden Rod. More than 28 species scattered over the state. Produce a large amount of pollen which is thought by some to be one of the causes of hay fever. Horses have also been poisoned from eating plants of this genus.
148. *Erigeron* spp. Fleabane. Several species in the state. Reported poisonous.
149. *Bidens* spp. Beggar-ticks or Spanish Needles. Several species in the state. Fruits cause local irritation because of barbs present.
150. *Achillea millefolium* L. Yarrow. Common all over the state. Poisonous nature due to its action upon the blood vessels, especially in the viscera of the pelvis where hemorrhage is caused.
151. *Chrysanthemum leucanthemum* L. Ox-eye or White Daisy. Common in pastures and meadows throughout the state. Flowers produce irritation.

152. *Tanacetum vulgare* L. Common Tansy. Frequent along roadsides and escaped from gardens. Very poisonous to animals, producing a condition similar to rabies. People are often poisoned.
153. *Artemisia absinthium* L. Common Wormwood. Occasional in waste places. The volatile oil of this plant in large doses produces cerebral disorders and convulsions. In horses there is produced a paralysis of the hind quarters.
154. *Arctium minor* L. Lesser Burdock. Common in all parts of the state. The fruit causes mechanical injuries, and the root has diuretic properties.
155. *Cirsium* spp. Thistle. Several species in the state. Spiny fruit and leaves cause inflammation.
156. *Anthemis cotula* L. May-weed. Common in fields and waste places throughout the state. Causes blistering of the skin in people.

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## THE PENETRATION OF CATIONS INTO LIVING PROTOPLASM <sup>1</sup>

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The following experiments on permeability are based mainly on a physiological reaction remarkable for its delicate balance, which on that account may reveal processes beyond the reach of quantitative chemical tests. The reaction in question is a contraction along their long axes of the chloroplasts and cytoplasmic strands in the cells of *Spirogyra* (Osterhout, 1916; Chien, 1917; Scarth, 1922, 1923, 1924). The mechanism of these movements has been discussed elsewhere (1924). With most polyvalent cations the response is extremely sensitive. If therefore one is able to prove that ions must penetrate before they can produce it, the reaction will afford a delicate qualitative test of permeability, with the further possibility of quantitative comparisons.

Proof, as far as it goes, is provided by a microchemical test in the case of Ba, whose action is the subject of many of our experiments. If filaments of *Spirogyra* which have responded to a Ba salt be washed and treated with  $H_2SO_4$ , the polarizing microscope usually reveals a fine crystalline precipitate in the protoplasm which is not present in a control that has not reacted to Ba. The chemical test, however, is less delicate than the physiological reaction and so can not prove the penetration of Ba to the limit of its effect. By comparison with Ba, those ions such as Ca whose penetrability <sup>2</sup> is known to be weak produce no effect, though in colloidal activity they may be practically equal to Ba (see later).

The facts that there is always a lapse of time between the application of the agent and the beginning of the reaction, and that this interval may vary from a few minutes to several hours according as the conditions modify permeability, point also to penetration of the agent as a necessary antecedent to its action. Just as in the case of cells of voluntary and heart muscle two types of ionic action are distinguished, a superficial and an internal, de-

<sup>1</sup> The cost of the chemicals used in this research was defrayed by a grant from the Honorary Research Council for Scientific and Industrial Research of Canada.

<sup>2</sup> "Penetrability" is used throughout this paper in the active sense, referring to the ions, "permeability" in the passive sense referring to the protoplasm, without, however, implying that the protoplasm is passive in the process.

pending on time relations, so the writer has observed two types in *Spirogyra*: a superficial change which begins almost immediately and is little or not at all influenced by factors that govern permeability (1923), and the internal change now considered which requires time and is profoundly influenced by these factors (1924). The time interval is greatly reduced by the following conditions:

(1) Freedom from all traces of Ca in the solutions, to attain which the purest salts are used and the distilled water is condensed in silica.

(2) Previous thorough washing of the biological material especially at or above 20° C.

(3) Maintaining the solutions at a similar, moderately high temperature. For example, material which at 23° C. contracts in BaCl  $10^{-3}$  M after five minutes, at 17° C. requires 30 minutes.

It is to be noted that the reaction except in its later phases represents normal protoplasmic contraction within a healthy cell, and as such is a criterion of the permeability of healthy and not of moribund protoplasm.

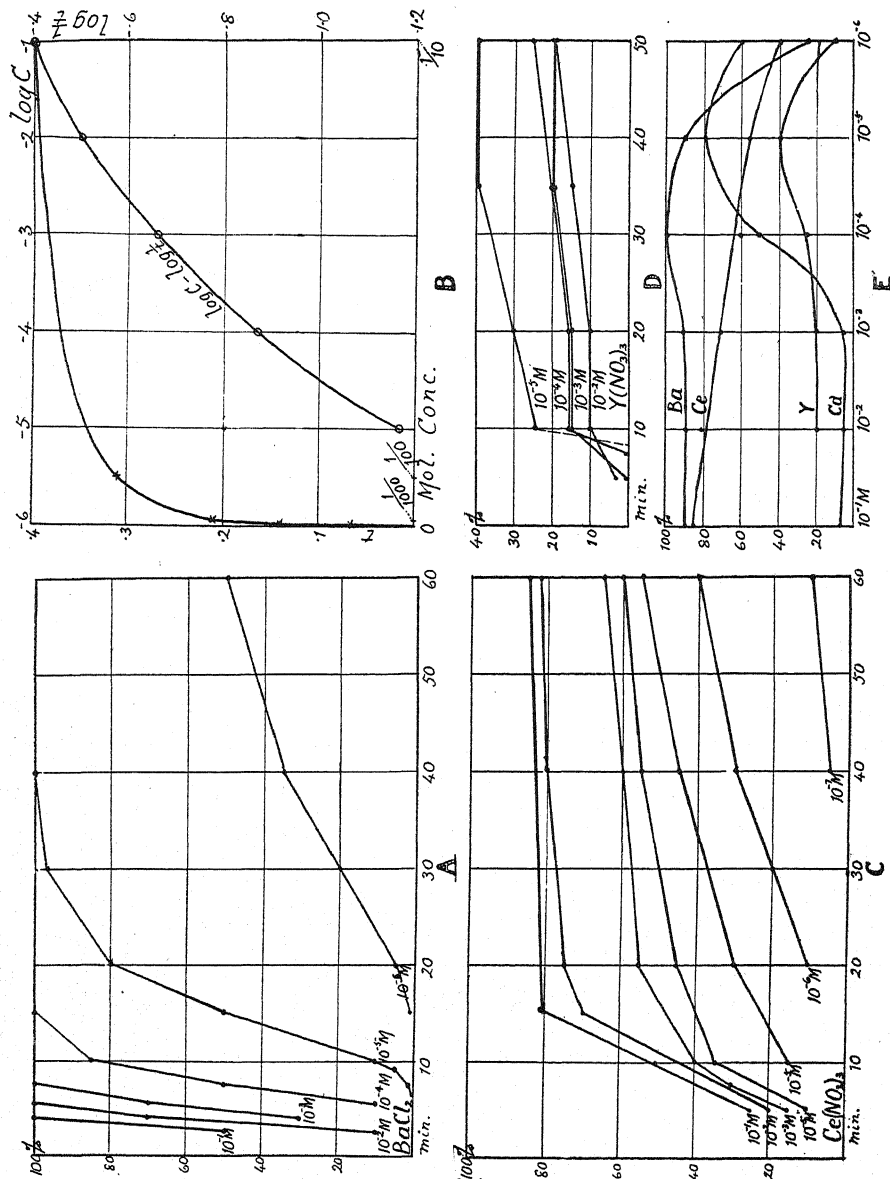
With these premises one may draw certain conclusions regarding the penetration of cations from the following experiments.

#### PENETRATION AS RELATED TO EXTERNAL CONCENTRATION

Evidently a certain minimum amount of any agent must penetrate to initiate contraction. Call this amount  $a$  and the time taken for it to penetrate  $t$ . The *rate of penetration* is equal to  $a/t$ . The quantity  $a$  is unknown, but for the same ion and under the same conditions it probably remains approximately constant at least within a certain range of penetration time.

The interval between the first application of an agent and the first appearance of contraction is the sum of the penetration time  $t$  and an unknown constant, the physiological "reaction time." The latter must be very brief, for contraction may begin in 2 minutes and of this period a large part must have been occupied in penetration. When, therefore, the time interval is many times this amount, the fraction of the whole that is reaction time becomes negligible and we may treat the observed interval as penetration time  $t$ . Since  $t$  is measurable and  $a$  is approximately a constant, we can compare the rate of penetration with varied concentration of salt.

Experiment: A few filaments of well washed *Spirogyra* are placed in a series of concentrations of BaCl<sub>2</sub> of uniform temperature with that of the bath from which they are taken. At frequent intervals they are quickly examined and the percentage of contracting cells is noted (table 2). To obtain a standard of comparison, the percentages recorded are plotted against time (text fig. 1, A) and from the curve the time required for 50 percent of the cells to contract is read off with considerable accuracy (table 1). Repetition with the same batch of biological material yields uniform results.



TEXT FIG. 1, B (table 1). Abscissae, external concentration; ordinates, rate of penetration;  $t$  = time for 50-per-cent contraction. Upper curve on natural scale; lower curve on logarithmic scale.

A, C, D. Abscissae, time since application of agent; ordinates, percentage of cells showing contraction. A (table 2).  $\text{BaCl}_2$  at  $23^\circ\text{C}$ . Spirogyra washed 24 hrs. C (table 4).  $\text{Ce(NO}_3)_3$  at  $23^\circ\text{C}$ . Spirogyra washed 24 hrs. D (table 6).

TABLE 1. (Text fig. 1, B.) Interval between application of  $BaCl_2$  at  $23^\circ C.$ , and 50-percent contraction in *Spirogyra porticalis*

Conc.	Time.	Conc.	Time
$10^{-1}M.$	2.5 min.	$10^{-4}M.$	7.5 min.
$10^{-2}$	3.2 "	$10^{-5}$	15.0 "
$10^{-3}$	4.6 "	$10^{-6}$	55.0 "

In text figure 1,  $B$ ,  $1/t$  is taken as the abscissa and corresponds to the rate of penetration if we regard  $a$  as approximately constant. The curve obtained resembles, but is not, a true adsorption curve, since, when plotted on the logarithmic scale, the points do not form a straight line.<sup>3</sup>

Two conclusions as to the nature of the absorption mechanism may be drawn from this result:

(1) It does not obey the ordinary laws of diffusion since the rate is not proportional to the diffusion gradient.

(2) It may involve *adsorption*, but not as the sole or limiting factor; there must be other complicating factors to account for the curvature in the logarithmic graph.

Tables 2-8 show the percentage of contracting cells in *Spirogyra porticalis* at intervals after first application of the stimulating agent.

TABLE 2. (Text fig. 1, A.)  $BaCl_2$  at  $23^\circ C.$ ; previous wash 24 hrs.

Min.	2½	4	5½	7½	10	15	20	30	40	60
$10^{-1}M.$	50	100								
$10^{-2}$	10	70	100							
$10^{-3}$	odd	30	70	100						
$10^{-4}$			10	50	85	100				
$10^{-5}$				1	10	50	80	97	100	
$10^{-6}$						1	5	20	35	50

TABLE 3.  $BaCl_2$  at  $23^\circ C.$ ; previous wash 4 hrs.

Min.	5	10	20	30	40	60
$10^{-2}M.$	50	60	75	85	90	90
$10^{-3}$	40	50	65	75	85	90
$10^{-4}$	10	40	60	80	100	100
$10^{-5}$	0	2	50	60	75	90

TABLE 4. (Text fig. 1, C.)  $Ce(NO_3)_3$  at  $23^\circ C.$ ; previous wash 4 hrs.

Min.	5	7½	10	15	20	30	40	60
$10^{-1}M.$	25		50	80				85
$10^{-2}$	20		45	70	75		80	82
$10^{-3}$	15	30	40		55			70
$10^{-4}$	10		35		45		55	60
$10^{-5}$			15		30		45	55
$10^{-6}$					10		30	40
$10^{-7}$							5	10

<sup>3</sup> We are dealing with rate of absorption and not with final equilibrium, yet if adsorption were the limiting factor which determined the rate, the latter would follow an adsorption curve.

TABLE 5.  $Y(NO_3)_3$  at  $20^\circ C.$ ; previous wash 30 hrs.

Min.	10	15	20	30	50
$10^{-1}$ M.....	2		70	90	dead
$10^{-2}$ .....			70	90	90
$10^{-3}$ .....			40	50	50
$10^{-4}$ .....			10	20	30
$10^{-5}$ .....			10	25	30
$10^{-6}$ .....				0	10

TABLE 6. (Text fig. 1, D.)  $Y(NO_3)_3$  at  $25^\circ C.$ ; previous wash 6 hrs.

Min.	5	$7\frac{1}{2}$	10	20	35	50
$10^{-2}$ M.....	3		10	10	15	20
$10^{-3}$ .....	1		15	15	20	20
$10^{-4}$ .....		1	15	15	20	25
$10^{-5}$ .....		0	25	30	40	40

TABLE 7.  $CdCl_2$  at  $23^\circ C.$ ; previous wash 1 hr.

Min.	15	30	50	75	100
$10^{-1}$ M.....	0	5	7	7	10
$10^{-2}$ .....	0	1	1	5	10
$10^{-3}$ .....	0	0	1	5	5
$10^{-4}$ .....		10	20	50	70
$10^{-5}$ .....	10	30	50	80	95
$10^{-6}$ .....		5	35	60	70

TABLE 8.  $SrBr_2$  at  $23^\circ C.$ ; previous wash 24 hrs.

Min.	15	60
$10^{-1}$ M.....	2	20
$10^{-2}$ .....	1	3
$10^{-3}$ .....	1	1

A study of the curves in text figure 1 and of the accompanying tables shows that complicating factors do exist and throws light on their nature.

In the case of Ba, when the conditions are favorable for rapid penetration (table 2, text fig. 1, A) there appears, as time passes, a falling off, though slight, in the rate at which cells become affected. This is shown to a much greater extent when the rate of penetration is slower (table 3) and better by trivalent Ce (table 4, text fig. 1, C) than by Ba. In such cases, the rate diminishes to zero before 100-percent contraction is attained. (Later on as the cells become moribund some further contractions may appear.) It will be observed in text figure 1, C that the curves of the higher concentrations begin to flatten out earlier than those of the lower, so that, if prolonged, the latter might eventually overtop the former. This sometimes happens with Ce even within the hour's limit.

In the case of Y (trivalent) the curves fall away still more quickly, and it is usual for the maximum effect to appear comparatively low in the series (table 6, text fig. 1, D). This is exhibited still more strikingly by Cd

(table 7, text fig. 1, *E*), which gives a high maximum about  $10^{-5}$  M and almost zero effect above  $10^{-4}$  M.<sup>4</sup>

The percentage of cells contracting at the end of an hour in the above-described experiments is shown graphically in text figure 1, *E*. The ascendancy of the lower concentrations becomes much greater if we take into account the average degree of contraction as well as the number of contracting cells.

The falling off as time proceeds of the curves of percentage contraction are no doubt in part due to the type of variability naturally exhibited by the cells, an asymmetrical sigmoid form being characteristic for the time curve of biological material whose variability presents a skew frequency curve. This alone, however, can not explain all the relations, especially the tendency in some cases for the high concentrations to fall away more than the low. It looks as if the salt itself were partly responsible for the falling off—a theory supported by the fact that ions inhibit one another's action according to the same laws as those governing their apparent self-inhibition (see later). That it is their own absorption rather than the contractile reaction which is interfered with is definitely indicated by the relative viability, if allowed to remain in the solution, of those cells which at the start are strongly and those which are slightly or not at all affected. The former quickly die, the latter may remain alive for days. Such an extreme difference in resistance is scarcely compatible with equal permeation of the toxic agent.

The inhibition of absorption is not due to saturation of the cell, for the stage at which it becomes manifest depends on the time of exposure to the salt and on external conditions but not on the amount absorbed.

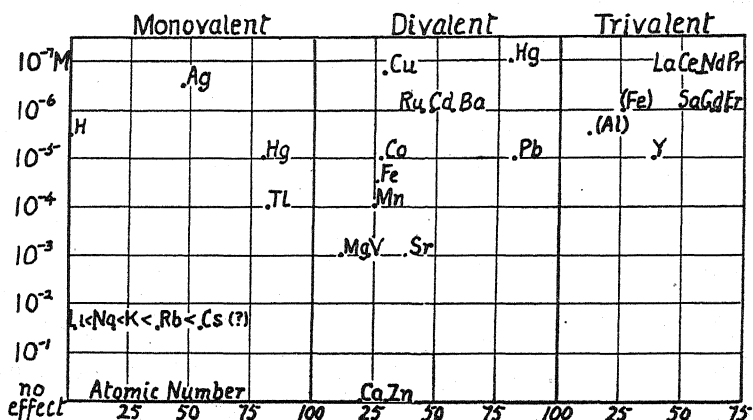
#### PENETRATION AS RELATED TO THE CHARACTER OF THE ION

In the case of the majority of cations, the reversible chloroplastic contractions referred to above can be used as a criterion of penetration. Concentrations that are toxic within the limits of the experiment are disregarded except in the case of the alkali metals to be discussed later.

It is necessary to know something of the relation of the contraction response itself to the various ions before we can use it to compare the relative speed with which they penetrate the cell. In former papers (1924) evidence has been adduced to prove that the reaction is quite comparable to similar changes in a physical emulsoid and follows the same laws. It was demonstrated that the axial contraction is due to the anisotropic structure of the contractile protoplasmic gel, and that it is invariably correlated with a fall in viscosity, just as volume changes in an emulsoid gel and viscosity changes in its sol vary alike in relation to the action of electrolytes. Since these changes in a physical emulsoid are sensitive to ions

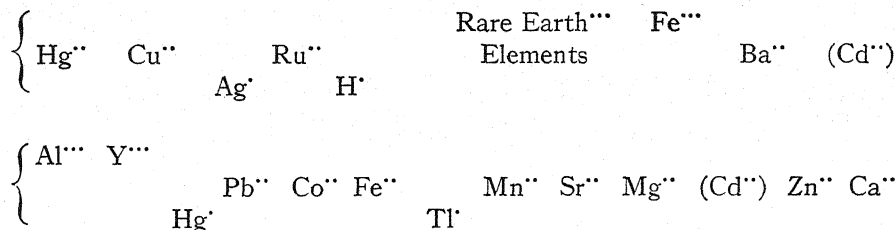
<sup>4</sup> The reaction to Cd salts is of a somewhat different character as compared with the others.

according to the law of valency—with a special activity on the part of the noble metals—we might expect the same to hold for the physiological reaction. In text figure 2 the various cations are classified according to the lowest concentration at which they cause contraction within an hour (it usually occurs within one hour or not at all). The general tendency obviously is to follow the above-stated law. There are several notable exceptions, however, but the significant point is that most of these, as will be shown presently, are ions whose penetrability is extremely slow. It seems justifiable, therefore, as a working hypothesis to assume that the valency law does hold as regards contraction effect and that apparent variations from it are due to difference in penetration capacity. Assuming this, it is possible to make deductions as to penetrability where it has not been determined by direct methods, and to discover certain general relations between the penetrability of ions and their physical properties.



TEXT FIG. 2. Cations arranged according to (1) lowest concentration producing contraction, (2) valency, and (3) atomic number.

A strict comparison of the relative speed with which ions cause contraction is difficult in view of the peculiar and divergent effects both of the concentration factor and of external conditions. Under the most favorable conditions for penetration the following order holds generally, the anion of the salt being Cl or  $\text{NO}_3$ :





The last two are indefinitely slow except as regards high (acid) concentrations of Zn. Cd occurs twice because, though very active in low concentrations, it has little effect in high. The alkali metals will be discussed later. The valencies are kept distinct so that comparison may be made of ions of equal colloidal activity within which limits, according to our preliminary hypothesis, the order is determined by penetrability. I shall now amplify the evidence in support of this hypothesis.

A critical examination of the above series or of text figure 2 shows that departures from the rule of colloidal activity follow a suggestive regularity. The level of the ions in the table so closely approximates their order in the speed series that I may use the former classification to illustrate this point. The figure shows the relation to atomic number (or atomic weight) as well as to valency, and it can be seen that the power of the ion to produce contraction tends to decrease with decrease of atomic number, becoming zero in Ca and Zn. It is only within separate groups of the periodic table that this rule strictly holds, and only by considering a whole range of concentrations is the *modus operandi* of the atomic-weight factor brought out.

Of the various chemical groups, only a limited number present a series of elements which are available for physiological comparison. Hydrolysis, insolubility, etc., of their salts exclude the others. The tables on pages 136 and 137 give the most important data regarding the available divalent or trivalent series. The monovalent alkali metals will receive separate discussion in a later paragraph.

Taking first the Ba-Sr-Ca group, Ba, besides having a lower limiting concentration than Sr, is swift and sure in its action whereas Sr is slow and uncertain and more dependent on favorable conditions of penetration. Ca has no effect. Similarly in the Hg-Cd-Zn group, Hg acts with extreme rapidity, Cd more slowly and to a marked degree only in *low* concentrations, there being merely a slow toxic action above  $10^{-4}$  M. Zn has no effect. Whence it is evident that Ca and Zn, in spite of their apparently anomalous position, really differ no more from Sr and Cd than do the latter from Hg and Ba respectively. In another divalent group Ru may be compared with Fe or Co.

Among trivalent ions a comparison is possible between the heavy rare earths and the lighter yttrium, but the salts of scandium, the third and lightest member of this series, hydrolyze too freely. It is seen that Y behaves like the other intermediate elements in that its action is slower and more uncertain than that of the heavier ions, with a tendency to be arrested before proceeding very far.

That these differences in activity among ions of the same chemical group and of similar colloidal activity are due to differences in their penetrating power is indicated by various independent and direct measurements. Reference has been made to a microchemical test which demonstrates rapid

penetration of Ba in the early stages of its contact with the cells. Sr on the contrary has been shown by Brooks (1921) to enter the cell sap of *Nitella* only slowly, while the penetration of Ca according to Osterhout's direct measurements can not be detected.

The salts of Hg and other noble metals are well known to penetrate cells with great rapidity. Cd and Zn are much less toxic to *Spirogyra*, sensitive though it is, than to some other organisms. In fact, Zn salts from  $10^{-2}$  M downwards (higher concentrations are too acid) are more favorable to the continued existence of *Spirogyra* than any other single salt except those of Ca and Sr. The inference is that Zn resembles them in non-penetration. Stiles and Kidd (1919), using 0.02 M concentration of  $ZnSO_4$ , found that absorption by pieces of carrot is initially slow but increases with time, owing, as they well suggest, to H-ion toxicity.

Among trivalent ions the absorption of Al—which resembles Y, though its salts are more largely hydrolyzed—has been studied by Rothert, Stoklasa, and others. The amount taken up by roots from weak concentrations of salt in soil or water cultures is found to be considerable, but decreases, not merely relatively but absolutely, as the external concentration is increased (from 0.001 N to 0.015 N; Stoklasa, 1922), just as the curve of contraction would lead us to expect.

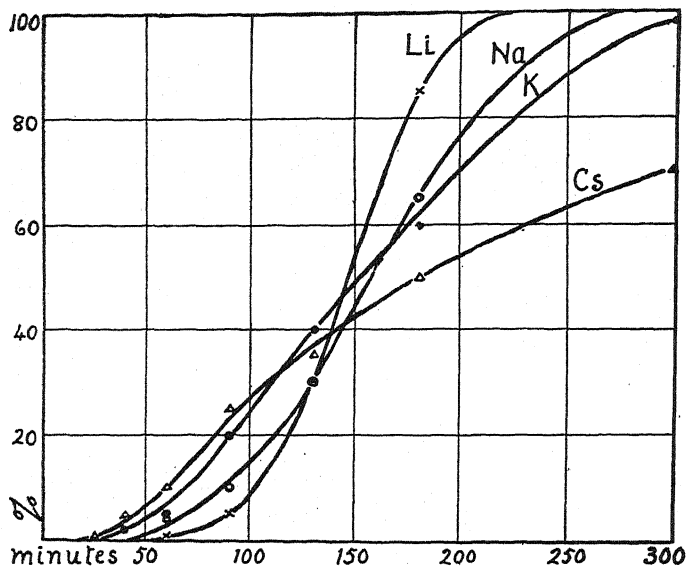
Such direct measurements of penetrability as are available, therefore, justify the interpretation of our indirect physiological test as a criterion of relative permeability for ions of equal colloidal activity, and support the statement that in any group of the periodic table the rate and amount of absorption of polyvalent cations at least for a limited time increases enormously with atomic weight. Further confirmatory observations will be quoted after treating of the alkali metals.

*Alkali Metals.* As a physiological test of the entry of these ions it was necessary to use any change, moribund or otherwise, that might appear in the cells, since normal contraction, while it may occur, especially with the heavier ions, is so commonly followed or replaced by other changes resulting from injury that alone it can not be used for measurement. The curious result appeared that, while the ultimate order of toxicity of the ions is Li . . . Cs, the rapidity with which they produce the first changes in the cells is according to the reverse order Cs, Rb, K, Na, Li. Table 9 and text

TABLE 9. (Text fig. 3.) *Penetration of alkali metal ions; percentage of cells of Spirogyra dubia affected by M/10 chlorid at intervals after first application*

Min.	25	40	55	90	130	180	300
Cs. ....	1	5	10	25	35	50	70
K. ....	0	2	5	20	40	60	98
Na. ....	0	0	5	10	30	65	100
Li. ....	0	0	1	5	30	85	100 (all dead)

figure 3 give the percentage of affected cells at various intervals. In the early stages this is represented merely by contraction or by slight injury such as loss of turgor; in the later stages most of the affected cells especially at the Li end are dead. Apparently Cs . . . Li is the order of initial penetrability and Li . . . Cs that of toxicity. While it is possible that this crossing over of the curves in text figure 3 may be due to a measure of self-antagonism (*cf.* Fitting) possessed to a larger extent by the heavier elements, there is no marked falling off such as is exhibited by the polyvalent ions.



TEXT FIG. 3 (Table 9). Abscissae, time since application of agent (M/10 chloride); ordinates, percentage of affected cells.

The rule that penetrability increases with atomic weight seems therefore to hold with monovalent as well as with polyvalent cations, though the variation is slight as compared with the latter.

#### RELATIVE PENETRABILITY AS DETERMINED BY OTHER WORKERS

The direct chemical tests by Osterhout and Brooks of the penetration of ions into the cell sap of *Nitella* have already been referred to. The alkali metal ions tested penetrate fairly rapidly, Ca and Sr very slowly. Stiles and Kidd (1919), using pieces of carrot and measuring the conductivity of the surrounding solution (concentration 0.02 N at start), arrive at an initial order of absorption: K, Na, Ca, Li, Mg, Zn, Al; and a final order: K, Na, Li, Ca, Mg, Al. The position of Ca in the initial order is so much at variance with that obtained by other methods that one wonders whether adsorption

by the cell walls of the tissue may not be responsible for it. Fitting (1915), using the deplasmolysis method, which measures osmotic changes in the cell sap, gives the order K, Na, Li, Mg, Ca = Ba, the last two showing no penetration. Tröndle (1918) by similar methods reaches a conclusion approximating my own, *viz.*, Rb, K, Na, Li, Mg, Ba, Sr, Ca. Kahho (1921), by a tissue-tension method which also measures osmotic changes, arrives at the order K, Na, Li, Mg, Ca = Ba. Tröndle appears to have been the first to state expressly that the speed of penetration increases with atomic weight in the same group of the periodic table. My results give a wider foundation for this rule and also show that the order of magnitude of the variation may be not slight but enormous if *initial* rate of penetration is compared.

This last conclusion covers the only outstanding discrepancy between my results and those quoted above, specifically as regards the rapid absorption of Ba and Al which I find to take place for the first 10 to 50 minutes (depending on concentration). With respect to Ba, I have mentioned a direct chemical test which supports the physiological one; and as regards Al, Stoklasa and Rothert find it to be largely absorbed from dilute solution. Since the rate of absorption falls off rapidly with time and does not always increase with concentration, it is obvious that protracted experiments with high concentrations—as are most of those cited above—need not give results identical with those of a method which applies largely to low concentrations and short intervals of time. Moreover, there is no *a priori* reason for supposing that ions which are rapidly taken up by the protoplasm of a cell should be equally rapidly released again into the vacuole in a state of osmotic activity. The slight discordance between the results is therefore less surprising than the preponderating harmony.

#### PENETRATION AS RELATED TO ELECTROLYTIC SOLUTION PRESSURE OF THE METALS

The physiological significance of the solution-pressure series especially as regards toxicity was demonstrated by Mathews, but perhaps without sufficient regard for exceptions. For it is only in the upper part of the reversed scale, *i.e.*, where a metal ion takes up a negative charge with the greatest readiness, that the property of electro-affinity predominates over other electrical properties in physiological importance. In the lower part of the scale toxicity as often as not runs counter to the order of ions as given below, which is the inverse of the solution-pressure series (from Landolt and Börnstein's Tables, 1923). Ions of different valency are kept distinct as before.

Reversed										
s.p.										
series										
	Hg <sup>++</sup>	Cu <sup>++</sup>	(Ru <sup>++</sup> ?)	Fe <sup>+++</sup>	Pb <sup>++</sup>	Co <sup>++</sup>	Cd <sup>++</sup>	Fe <sup>++</sup>		
			Ag <sup>+</sup>	Hg <sup>+</sup>		H <sup>+</sup>	Tl <sup>+</sup>			
Cont'd										
	Zn <sup>++</sup>	Mn <sup>++</sup>	Al <sup>+++</sup> and certain rare <sup>+++</sup>	earth elements	Mg <sup>++</sup>	Ca <sup>++</sup>	Sr <sup>++</sup>	Ba <sup>++</sup>		
					Li <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>			

The general distinction as to the relative importance of the two parts of the scale also holds if it is compared with the order of speed in producing contraction (see series on page 139) or with the level in text figure 2 of the various ions. The earlier part of the series corresponds closely with the physiological order, as follows:

*Decreasing Order of Activity*

Fe<sup>+++</sup>  
 Hg<sup>++</sup> Cu<sup>++</sup> Ru<sup>++</sup> (Cd<sup>++</sup>) Pb<sup>++</sup> Co<sup>++</sup> Fe<sup>++</sup> Mn<sup>++</sup> (Cd<sup>++</sup>) Zn<sup>++</sup>  
 Ag<sup>+</sup> H<sup>+</sup> Hg<sup>+</sup> Tl<sup>+</sup>

The only discrepancies are the transposition of Mn<sup>++</sup> and Zn<sup>++</sup>, a slight rating down of the monovalent Hg<sup>+</sup> and Tl<sup>+</sup>, and the doubtful position of Cd<sup>++</sup>, depending as it does on concentration. Kahho (1921a) also found a tolerable correspondence between the penetration of the heavy divalent metals and their solution pressure.

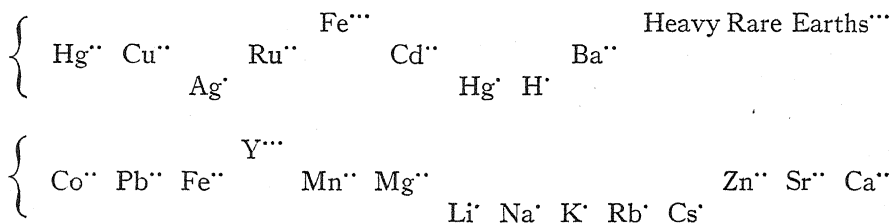
On the other hand, the physiological order of the ions that follow Zn is little or not at all determined by their electro-affinity. The trivalent rare-earth elements rank equal to the noble metals in power to produce contraction and only a little lower in speed of action. The promotion of Al is only a little less, while Ba leaps from the lowest position to one of the highest. In fact, the order of penetrability among the alkali, alkali-earth, and rare-earth elements is in general the reverse of what we might expect if the solution pressure rule prevailed. As we have seen, the dominant factor in each group is atomic weight or atomic number. This rule applies even to the earlier part of the inverse solution-pressure scale, for there the two factors are in harmony and not, as in the latter part, opposed to each other.

#### PENETRATION AS RELATED TO GENERAL PHYSIOLOGICAL ACTIVITY OF THE ION

That there is a general order of physiological activity of metal ions is well recognized. It may vary somewhat for different organisms, but generally the same order holds if the ions are classified according to their least toxic and their least stimulating concentrations. The vital functions which have been mostly studied in this regard are respiration, growth, and reproduction. Mention may be made of papers by Mathews (1904) already cited, True and Gies (1903), Livingston (1905) (including a comprehensive summary of literature up to date), Kahho (1921c), and Hotchkiss (1923).

To the many physiological activities which have been found to be stimulated by metal ions I may add that of protoplasmic streaming. Seifriz (1923) found that Cu, Ba, and to a less extent Sr promote cyclosis in *Elodea*. As he argues, this can hardly be explained merely by a fall in viscosity, for this could not *initiate* streaming in a cell at rest. I find similarly that vigorous streaming movements are set up in *Spirogyra* by Cu, Li, Ce, and Ba, but not by ions of low physiological activity.

The present question is whether the absorption of ions follows the same order. Kahho has recently endeavored—with some success as regards the divalent heavy metals—to correlate toxic activity and penetrability into the cell sap. With respect to Spirogyra it has been shown above, using the speed with which contraction is produced as a rough index of penetration speed, that this corresponds closely with the inverse of solution pressure at least towards the more active end of the series, which in turn agrees with the order of toxicity to organisms in general. The same toxic order applies to Spirogyra. The lowest concentration that is toxic within an hour is generally a little higher than that which just causes contraction, the order of decreasing toxicity being:



Where order of toxicity differs from that of penetrability, it is in the direction of a closer approximation to the solution-pressure series.

The most notable discrepancy between the two physiological series is seen in the alkali group where initial penetration and toxic action vary in opposite directions. Neither, however, varies greatly—in striking contrast to other groups—nor is the order of toxicity so consistent for all organisms as it is in the other chemical series. Hence it is easier for the correlation between penetration capacity and general physiological activity to be overcome by other ionic properties, such as adsorbability or speed of migration, which may also affect rate of penetration. Apart from this, the only significant divergence between the toxic series and that based upon speed in producing contraction is the higher position occupied by trivalent ions in the latter. No doubt this is mainly due to the fact that less is required to cause the reaction, but it is possible that valency has a significance in absorption also which it does not have in physiological stimulation in general.

In terms of the modern electronic theory it may be said that at least three different electrical properties of metal ions determine their physiological activity including penetrability: *viz.*, valency or the number of “free” electrons, atomic number or the number of “bound” electrons, and the attraction of the ion for electrons.

Since it is impossible in the present state of our knowledge to explain the physiological series of ions in terms of any single physico-chemical law and still less to arrive at a single physical mechanism underlying the many diverse physiological activities that may be stimulated in accordance with it, we can describe the absorption of ions in protoplasm, and probably also

their passage through it, only as a "vital function." The only hints as to mechanism are the resemblance of the curve of penetration *versus* concentration to an *adsorption* curve, and the possible tendency for colloidal activity or *adsorbability* to render ions more active in initial penetration than their position in the scale of general physiological activity would indicate. Stimulation by physiologically active ions results in the speeding up of the absorptive function. Possibly, though not necessarily, other substances in contact with the cell as well as the stimulating agent might thereby be taken up with increased rapidity, but this has yet to be proved. The theory that protoplasm performs work in the uptake of substances is favoured by the relation of absorption to heat energy, light energy (Hoagland and Davis, 1923), and general functional activity. This is of course an old view of the process and one that has recently been argued by Tröndle (1918). This author also regards the falling off in the rate of absorption as a fatigue phenomenon. But there are other possible causes, and one that can be proved to exist is the antagonistic action of ions, which must now be considered.

#### THE ANTAGONISTIC FACTOR

Evidences of some action of ions or salts which antagonizes their own penetration have already been noted, *viz.*, the falling off in the rate of penetration and the flattening out or occurrence of a maximum in the curve of penetration *versus* concentration. That these phenomena are of the same nature as the action of one ion in antagonizing the entry of another is fairly well proved by the fact that they follow the same laws. It will be convenient to speak of auto-antagonism and hetero-antagonism to distinguish them.

The experiments on the subject will be published separately, but some of the results may be briefly stated here.

(1) They entirely agree with Osterhout's conclusion that the antagonistic property is possessed equally by all divalent ions, and to a much greater extent but equally among themselves by the trivalent ions. Such a conclusion will be reached, however, only if the greater toxic tendency of the heavier ions in each group is in some way guarded against or discounted. The only monovalent ions found to exhibit any pronounced antagonism to Ba contraction (*i.e.*, to penetration) were Cs and Rb, though viability is greater in a suitable mixture of K or Na also with any polyvalent cation than with the latter alone.

(2) The curves on page 135 show that the auto-antagonistic effect does not immediately display its full power of inhibition, but takes from 10 to 60 minutes—depending on the concentration—to reach a maximum. Experiment shows that hetero-antagonism behaves in the same way.

(3) The condition of the cell also—as affected by previous treatment—determines its ability to react to both auto- and hetero-antagonism in

exactly the same fashion. Prolonged washing reduces the tendency for the rate of penetration to fall off, for the reaction to proceed farther in high than in low concentration, and for the inhibition of one ion's action by another. This is especially the case if the temperature of the bath is kept fairly high— $20^{\circ}$  to  $25^{\circ}$  C.

As regards the mechanism of antagonistic action, some interesting correlations are to be noted. Hansteen-Cranner's (1919) discovery that the lipid which permeates the cell wall of growing tissue is leached out in the absence of calcium (water-soluble lipoids at low temperature and non-water-soluble lipoids at higher temperatures) affords a suggestive parallel to the effect just cited. Another correlation is that between the antagonistic action of ions and their effect in producing adhesion between protoplasm and cell wall. Divalent ions produce a slight, and trivalent ions a powerful, adhesion (Stoklasa, 1911, 1922; Szűcs, 1912; Scarth, 1923). This effect, like decrease of permeability, is largely prevented if the cells have been well washed previous to treatment with the trivalent ions. Taking all the facts together, it appears probable that leaching removes some substance on which the ions act to produce the changes in permeability and cohesion. Hansteen-Cranner's work has done much to establish the predominantly lipid nature of this substance, without however precluding the possibility that other substances also play a part. Exactly what the change may be which takes place in the protoplasmic exterior and in the cell wall under the action of the colloiddally active ions is not yet apparent. Kahho suggests coagulation, and in support of this view I may instance a certain rigidity and brittleness of the cell walls that is induced by trivalent ions. It is, however, rather difficult to understand how an impermeability thus produced should disappear on death, which itself involves coagulation.

#### SUMMARY

A reaction in the cells of *Spirogyra*, which is shown to require penetration of the stimulating agent, is used as a criterion of the penetration capacity of cations.

Penetration of many divalent and trivalent cations is rapid at first but soon slows down, apparently to a standstill.

The curve obtained when initial penetration is plotted against external concentration at first rises steeply and then flattens out or even falls away in successively higher concentrations.

The penetrability of an ion is determined by two distinct and opposing reactions of the cell, one tending to active absorption of the ion, the other to its exclusion. The latter takes some time to reach its full power, in which interval the former displays its maximum but gradually decreasing activity.

The sensitiveness of both reactions increases with the valency of the cation; the former, but not the latter, is markedly affected by other properties.



The factor of active absorption increases greatly with the atomic weight of the cation in any particular chemical group. It also increases inversely with solution pressure, but only at the heavy-metal end of the solution-pressure series.

The order of initial penetrability of ions is mainly that of their general physiological activity, so that active absorption is apparently a "vital function."

The mechanism of the self-antagonizing action of ions appears to be identical with that of antagonism between ions, or at least with one type of such antagonism. Depending simply on the colloidal activity of the ion, it has probably a simpler mechanism than the absorption factor. The seat of it appears to be the lipid exterior of the protoplasm, since leaching of these lipoids (Hansteen-Cranner) reduces the capacity of the cell to react to antagonistic action of ions.

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# THE INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE DEVELOPMENT OF THE POLLEN TUBE OF THE SWEET PEA (*LATHYRUS ODORATUS*)<sup>1</sup>

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That the pollen grains of various plants might be induced to germinate under artificial conditions has been known for a long time. Several investigators have adopted this mode of attack in seeking to determine the requirements for pollen-tube growth. Extending the earlier findings of Bobilioff-Preisser (2) and Knight (5) with *Vinca minor* and the apple respectively, the present writer (Brink, 3) has shown that pollen tubes of *Scilla*, *Chionodoxa*, *Puschkinia*, *Muscari*, and *Vinca* equal in length to those growing in the pistils of these forms may be secured on a culture medium consisting of cane sugar, sterile yeast, and agar. It was demonstrated also that, in the *Scilla* tubes grown under artificial conditions, the nuclei were in position to function had fertilization been possible. These results show that pollen-tube growth, constituting as it does an essential link in the process of sexual reproduction among the higher plants, may be imitated *in vitro*, at least in regard to the prime factors of length of tube and nuclear distribution. They lend support to the view, moreover, that this method of approach may ultimately furnish the facts necessary to an interpretation of the physiological processes involved and throw light upon certain related phenomena of primary interest in plant genetics.

From the results of the studies noted above and others, various helpful conclusions have been drawn. The methods employed, however, left much to be desired from the quantitative standpoint. With the media used, an adequate knowledge of the conditions of the experiments was often impossible. These experiments served, however, for the discovery of some of the more important variables and made it clear that further progress was dependent upon the development of a methodology that would permit of their effective control. Only under such conditions could a precise measurement of the various factors contributing to growth be made.

The method described here for determining the effect of hydrogen-ion concentration on the development of pollen tubes is based upon the results of experiments on the influence of neutral salts. While the writer hopes to publish these results shortly, brief statement of some of the findings must be made at this time, since it was only through a knowledge of the effects of

<sup>1</sup> Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, no. 41. Published with the approval of the Director of the Station.

certain other ions that it was possible to develop a technic that permitted the measurement of the effects of active hydrogen.

As is well known, in order that the concentration of hydrogen ions in a solution be varied, the concentration of some other ion must be correspondingly altered. If this latter ion has an appreciable physiological action, and this is not measured or controlled, it may be impossible to dissociate the effects of the two variables introduced into the culture medium. The investigations alluded to above showed unmistakably that pollen is extremely susceptible to the toxic influence of various neutral salts. It was found that it is the cation which induces the striking physiological effect. If the anion is active, its influence is of another order. A real difficulty was thus encountered in arranging an experiment whereby the influence of the hydrogen ion could be determined; the substances added to change its concentration were in themselves so toxic that germination was inhibited.

A way out of the *impasse* was found with the discovery that calcium, at least in the case of sweet-pea pollen, behaved differently from the other cations tried. While in a medium otherwise favorable for growth, potassium, sodium, and lithium salts prevented germination at concentrations near M/100, and magnesium and barium salts were equally toxic at about M/1500, calcium salts in concentrations ranging from M/50 to M/500 considerably enhanced the growth of the pollen tubes. It was found, moreover, that in the presence of suitable concentrations of calcium salts the very toxic effects of the monovalent cations, sodium and potassium, were not in evidence. This opened up new possibilities in the study of the influence of electrolytes upon pollen-tube growth.

#### METHODS

Throughout the experiments to be described sweet-pea pollen was used. It can readily be obtained in good condition, and it germinates and grows well in suitable artificial media. Strong pollen tubes may be secured in sucrose solutions ranging in concentration from 10 percent to 30 percent. A 25-percent solution formed the basis of the culture media here used. The advantage gained in using a relatively high concentration of sugar lies in the fact that it lowers very appreciably the amount of bursting, a potent factor in determining the ultimate length of tubes. This phenomenon has been discussed by Brink (3) elsewhere.

Two series of experiments were made. In the first, the pH was adjusted with KOH and  $H_3PO_4$ . This will be called, for convenience, the phosphate series. Since potassium is so toxic that an M/100 solution of one of its neutral salts inhibits germination and growth, the concentration of this ion was kept constant in all the solutions in this series and an amount of  $CaCl_2$  was added such that the culture media when finally made up contained KOH and  $CaCl_2$  in the concentrations M/200 and M/100 respectively.

Previous studies had shown that the toxic effect of the potassium ion could in this way be almost if not entirely prevented. In order to determine the proportions giving the desired hydrogen-ion concentrations, 5-cc. samples of an M/25 KOH solution were titrated with approximately M/25  $\text{H}_3\text{PO}_4$  in the presence of 2 cc. M/5  $\text{CaCl}_2$  and of the appropriate indicator, against standard buffer solutions prepared according to Clark (4). The required amounts of acid having been obtained, each was added to 5 cc. M/25 KOH and 2 cc. M/5  $\text{CaCl}_2$  and made up to 20 cc. When mixed with an equal volume of 50-percent sucrose solution they were used as culture media.

While their initial hydrogen-ion concentrations could be adjusted closely, these media were less desirable in other regards. First, the pH range could not be carried into the alkaline zone on account of the precipitation of calcium phosphate. About pH 6.5 appeared to be the upper limit in that direction. And, secondly, because of the relatively weak buffer effect of the phosphate system over an important section of the range covered, grave doubts were held as to the stability of the solutions during the growth period. The further possibility existed that the phosphates might be taken out of solution by the pollen tubes, thus occasioning further disturbances in the hydrogen-ion concentration.

In the second series of experiments the culture solutions contained 3 percent gelatin, the buffer effect of which was utilized in adjusting the pH with HCl and KOH. We shall designate this the gelatin series. A very highly purified gelatin (0.045 percent ash), isoelectric or nearly so, procured from the research laboratory of the Eastman Kodak Company, was employed.  $\text{CaCl}_2$  was again used to protect the pollen tubes from the toxic effect of the potassium in the media.

Two methods of making up these culture media presented themselves. To a solution containing a known amount of isoelectric gelatin, sufficient standard KOH might be added to carry it well into the alkaline range, and the pH adjusted by adding successively increasing amounts of standard HCl. Or, secondly, the desired pH values might be obtained by adding varying amounts of standard HCl on the one hand and KOH on the other to the isoelectric gelatin. In the former case the concentration of the toxic potassium would be constant in all the culture solutions, and in the latter it would be variable above pH 4.7, the isoelectric point of gelatin, and absent in the solutions below that value. The question arose whether the concentrations of potassium necessary for the higher values of pH might exceed the protective effect of M/100  $\text{CaCl}_2$ . The amount of potassium, other conditions remaining the same, that might be added to a sugar medium containing M/100  $\text{CaCl}_2$  before a decrease in pollen-tube growth resulted was not definitely known. Some previous experiments with KCl indicated, however, that the concentration lay between M/50 and M/25, possibly near M/40. About M/45 was the concentration of KOH necessary to bring a 3-percent solution of isoelectric gelatin to pH 10.0, the upper limit in this

series; and, since the cultures were somewhat more easily prepared, this latter method was adopted.

In view of the toxicity of minute amounts of most cations, particular effort was made to obtain pure chemicals for these studies. The distilled water on tap in the laboratory was again distilled into Pyrex glass before being used, the usual apparatus employed being stoppered with cotton plugs. The pollen was grown in drop cultures on cover glasses inverted on pitted slides and sealed with vaseline. During the period of growth the cultures were kept in a well regulated constant-temperature chamber set at 25° C.

A period of from five to eight hours was required for the tubes to reach their maximum length. While sterile conditions were not observed, precautions were taken to avoid disturbances on this account. Fresh solutions and clean glassware were always used. In relatively few instances in our studies did the number of micro-organisms in the culture media become large enough during the short growth period to cause apprehension. Occasionally it appeared that the pollen was the source of a sufficiently large initial inoculum to cause irregularities. Such cultures were discarded.

Germination counts were made with the microscope under a magnification of about 200 diameters. Several fields in each drop were brought into view and the number of germinated and ungerminated pollen grains was recorded. Tube length was determined with an 8X ocular micrometer and an 8-mm. objective, giving a magnification of about 160 diameters. The pollen tubes of sweet pea follow a fairly direct course during elongation, and a satisfactory degree of accuracy can be obtained in their measurement if care is exercised.

## RESULTS

### The Phosphate Series

In the phosphate series determinations on percentage germination and amount of growth were made at intervals of 0.8 pH over the range pH 3.0 to pH 6.2. These tests were repeated 7 times, and in each set a check culture containing only 25 percent sucrose was included. The pH of this solution was about 5.8. This check culture, used also in the gelatin series, served as a means of detecting poor pollen and, moreover, as a common standard with which all the cultures might be compared.

The percentages of germination obtained in the 7 different tests are given in table 1. The average germination in the 7 check cultures, based on an aggregate of 1191 grains, is 85.5 percent. Tests 1 and 2 represent the extreme deviates, the former being 13 percent above the average and the latter (the only one dropping below 80 percent) is 20 percent below. In the remainder of the check cultures the fluctuations are not large, regard being had to the differences expected on the theory of simple sampling. It should be noted that the percentages of germination in the remaining cultures in test 2 are not low, relative to those of like composition in the

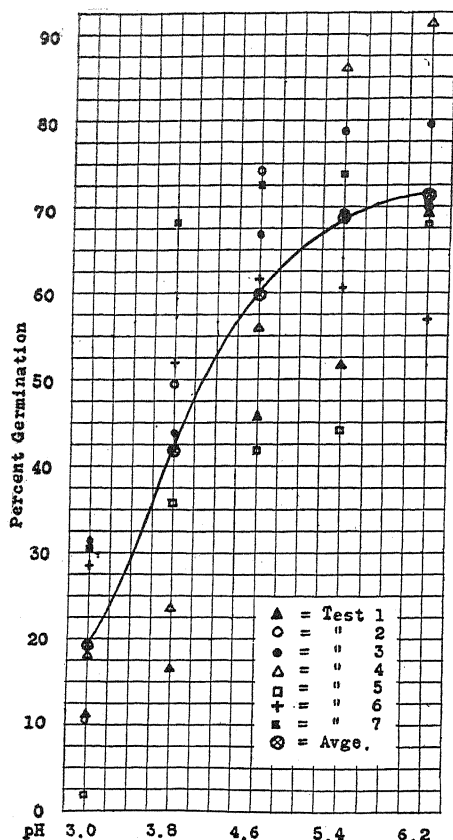
other tests. The conclusion seems warranted that the pollen used in these experiments was uniformly viable.

TABLE 1. *Percentage germination of sweet-pea pollen in cultures in the phosphate series ranging from pH 3.0 to pH 6.2. The results for 7 tests are listed separately, and the number of grains upon which each determination is based is given.*

Test	Check		pH 3.0		pH 3.8		pH 4.6		pH 5.4		pH 6.2	
	n	%	n	%	n	%	n	%	n	%	n	%
1.....	118	98.3	162	11.1	184	16.8	164	45.7	135	51.8	161	68.9
2.....	127	65.3	191	11.0	215	49.8	181	74.6	243	69.5	186	68.8
3.....	146	93.8	182	31.3	207	43.9	244	67.2	260	78.5	193	79.3
4.....	190	81.6	224	17.8	207	23.7	190	55.8	261	86.2	255	91.4
5.....	193	83.4	187	2.1	186	35.5	242	42.1	201	44.3	264	67.4
6.....	205	91.7	195	29.1	236	52.1	236	61.9	216	60.6	242	57.0
7.....	212	83.9	208	30.3	221	68.3	200	72.5	155	73.9	210	68.9

Examination of the amounts of germination obtained in the cultures in which an attempt was made to regulate the acidity reveals that, in general, the percentage germination rises steadily with increasing pH. There are some irregularities; in test 6 the percentage germination at pH 5.4 and pH 6.2 falls slightly below that at pH 4.6; in tests 2 and 7 the germination decreased somewhat at pH 6.2.

When the values obtained at a given pH in the several tests are compared, the variation is seen to be quite considerable. One is impressed with this fact when the results of the separate tests are plotted as shown in text figure 1. The mean values for each pH calculated on the basis of the respective totals are also plotted, and the points are connected. It is clearly evident that there is considerable variation around this line. An examination of the distribution of the values for the several tests affords some clue to the nature of this variability. In tests 1 and 5 all the points are below the average; in test 3, and in test 7 except at pH 6.2, all the points are above the average. It would appear that in these tests some constant factor entered, promoting or depressing the amount of germination, as the case may be, throughout each set. When the four successive increments in amount of germination in these tests are calculated, however, it is seen that the tendency to vary in both directions from the respective average increments characterizes the results in each test. In addition, then, to the above-mentioned source of variability, there is a considerable residuum of variation which we can ascribe only to chance. In the remaining three tests, namely, 4, 5, and 6, these chance fluctuations appear to be relatively more important. The increase in average percentage germination with increasing pH from 3.0 to 6.2 as shown in text figure 1 is very orderly, but undue emphasis should not be placed upon that characteristic of the curve.



TEXT FIG. 1. Percentage germination of sweet-pea pollen in the phosphate series. The curve passes through the mean for each pH. Around it the values for each of the 7 tests are plotted.

We should not have dwelt upon this point did it not serve to indicate that in experiments of this kind, even when all ordinary precautions are taken to eliminate systematic errors, the amount of variability in the results is still so great as to render conclusions based upon small amounts of data of questionable value.

Table 2 shows the amount of growth in length of the pollen tubes in the phosphate series. The number of tubes measured and their mean length in microns are tabulated separately for each of the 7 tests. Tests 1, 2, 3, and 5 show steady increases in mean tube length as the pH rises from 3.0 to 6.2. Similar increases are shown in test 4 from pH 3.0 to pH 5.4, but the amount of growth falls off at pH 6.2. Tests 6 and 7 exhibit further irregularities, the former at pH 5.4 and the latter at pH 5.4 and pH 6.2. Counter to the general behavior, these particular cultures fail to show increases in growth over all the preceding members in their respective series.

TABLE 2. *The mean lengths in microns of sweet-pea pollen tubes in cultures ranging from pH 3.0 to pH 6.2 in the phosphate series. The results for 7 tests are listed separately, and the number of tubes measured in each case is given.*

Test	Check		pH 3.0		pH 3.8		pH 4.6		pH 5.4		pH 6.2	
	n	Mean Length	n	Mean Length	n	Mean Length	n	Mean Length	n	Mean Length	n	Mean Length
1.....	47	303	12	38	31	105	49	206	52	196	54	244
2.....	61	79	28	85	45	158	55	163	44	180	44	212
3.....	47	272	44	85	59	144	60	157	59	170	60	195
4.....	52	114	29	63	40	129	44	182	52	221	55	155
5.....	57	328	4	65	32	118	55	139	40	177	52	239
6.....	56	191	41	80	49	120	47	199	47	176	53	277
7.....	49	292	31	97	53	157	49	220	46	173	49	183

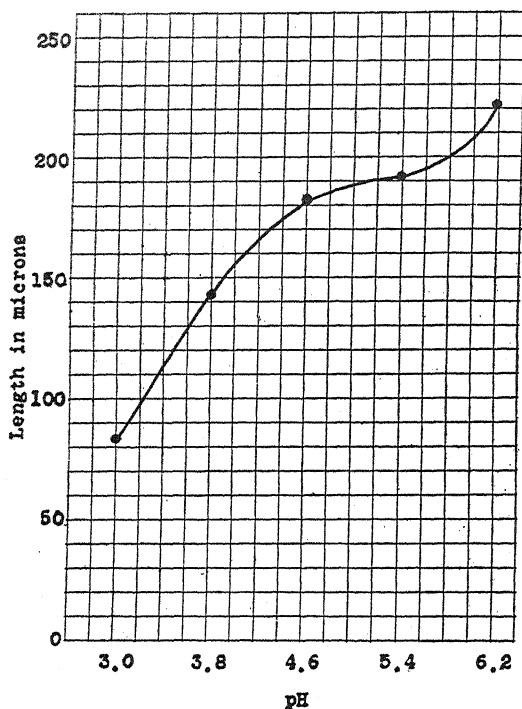
TABLE 3. *Summary Table of Pollen-tube Length in Microns in the Phosphate Series*

Culture	n	Mean	Standard Deviation	Coefficient of Variability
Check.....	369	203 $\pm$ 4.20	120 $\pm$ 2.97	58.82
pH 3.0.....	189	84 $\pm$ 1.28	26 $\pm$ .91	31.31
pH 3.8.....	309	142 $\pm$ 1.66	43 $\pm$ 1.18	30.51
pH 4.6.....	359	182 $\pm$ 2.10	59 $\pm$ 1.49	32.43
pH 5.4.....	340	191 $\pm$ 1.91	52 $\pm$ 1.35	27.36
pH 6.2.....	367	221 $\pm$ 2.03	80 $\pm$ 1.99	36.00

These data have been brought together in summary form in table 3. For each different culture medium all the data obtained in the 7 tests were thrown into 38.5 micron classes and the means, standard deviations, and coefficients of variability were calculated. In general, the frequency curves showed some skewness in the positive direction; only in the case of the check culture was this marked. The mean lengths for the five different media in which the pH was regulated are shown in text figure 2. Significant increases in growth occur as the pH of the media is increased from 3.0 to 3.8 and from 3.8 to 4.6. The average length of the tubes in the culture at pH 5.4 is  $9 \pm 2.83$  microns greater than that at pH 4.6. Considered in relation to its probable error this difference may not be significant. A further statistically significant increase occurs, however, at pH 6.2.

The variability in length, as shown by the standard deviation and the coefficient of variability, is high in all the cultures in the phosphate series. This is especially true in the case of the check culture, where the coefficient of variability is nearly 59. The least variability relative to the mean is shown at pH 5.4, but even here it is high. These facts add emphasis to our conclusion stated above, that in order to get an adequate statistical representation of the facts in experiments of this sort large amounts of material must be examined.





TEXT FIG. 2. The mean length of the pollen tubes in the phosphate series. Each point represents the average of 7 tests.

### The Gelatin Series

In the phosphate series it was not possible to study the germination and growth responses of the pollen above pH 6.2 on account of the precipitation of calcium phosphate in the media. By using gelatin as a buffer, however, media ranging from pH 3.0 to pH 10.0 by intervals of 1.0 pH were readily made up. It is a matter of regret that, after these cultures were developed, it was possible to make only two complete tests with them. But the work had to be laid aside at that stage through pressure of other duties. The data obtained, nevertheless, serve to extend materially our knowledge of the relation of pollen germination and pollen-tube growth over an important range of the pH scale, namely, that in the vicinity of the neutral point.

TABLE 4. *Percentage Germination of Sweet-pea Pollen in the Gelatin Series*

Culture	Check	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 10.0
Percent Germination . . . . .	93.2	0.0	48.5	77.0	81.0	74.9	40.8	28.3

Table 4 shows the percentages of germination obtained in the media in the gelatin series. The results in the two separate tests showed fairly close

agreement, and they have been averaged. The check culture, as in the phosphate series, contained only 25 percent sucrose. In the cultures where the hydrogen-ion concentration was adjusted, it was found that no germination occurred at pH 3.0 or at pH 4.0; about one half the grains germinated at pH 5.0; at pH 6.0, pH 7.0, and pH 8.0 over 75 percent germinated, the highest value, 81.0 percent, being obtained at pH 7.0. Farther on the alkaline side the amount of germination was distinctly lower. At pH 9.0 it decreased to 40.8 percent, and to 28.3 percent at pH 10.0. These relations are shown graphically in text figure 3.

A summary of the data on amount of growth in the cultures of the gelatin series is presented in table 5. At pH 5.0, the most acid culture in the

TABLE 5. *Summary Table of Pollen-tube Length in Microns in the Gelatin Series*

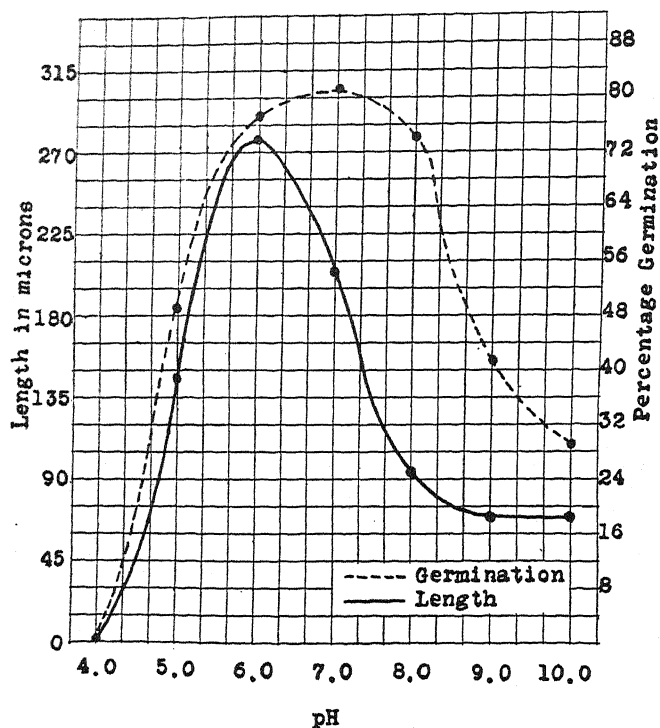
Culture	n	Mean	Standard Deviation	Coefficient of Variability
Check.....	92	298 $\pm$ 10.99	156 $\pm$ 7.78	52.49
pH 4.0.....	142	0	0	0
pH 5.0.....	81	148 $\pm$ 3.23	43 $\pm$ 2.23	28.87
pH 6.0.....	90	281 $\pm$ 7.78	110 $\pm$ 5.54	39.13
pH 7.0.....	111	208 $\pm$ 7.55	120 $\pm$ 5.46	57.67
pH 8.0.....	111	92 $\pm$ 2.16	34 $\pm$ 1.52	36.55
pH 9.0.....	78	71 $\pm$ 1.62	21 $\pm$ 1.14	29.79
pH 10.0.....	97	71 $\pm$ 2.85	42 $\pm$ 2.02	58.79

series permitting germination, the pollen tubes averaged 148 microns in length. A marked increase in growth is shown at pH 6.0, the most favorable medium in the set. A statistically significant decrease of 73 microns occurred at pH 7.0. A further sharp drop in average length amounting to 116 microns is shown at pH 8.0. As illustrated in text figure 3, the curve flattens very appreciably at this point, although the tubes at pH 9.0 averaged 21  $\pm$  2.7 microns less in length. The same amount of growth was obtained at pH 10.0 as at pH 9.0.

The variability in these cultures as indicated by the standard deviations and coefficients of variability is again very high. In general the variation is greatest in the cultures giving the highest average growth. It should be noted, however, that at pH 10.0 the coefficient of variability is 58.8, or nearly twice as great as at pH 9.0 where the average length of the tubes is the same. Possibly this difference is due to the greater concentration of potassium in the culture of higher pH. It will be recalled that the amount of KOH necessary to bring a 3-percent solution of isoelectric gelatin to pH 10.0 approaches, if it does not slightly exceed, the limit where M/100 CaCl<sub>2</sub> will no longer completely suppress the toxic effect of the K ion.

### Rate of Germination

Table 6 shows the rate of germination of sweet-pea pollen in gelatin media of different hydrogen-ion concentrations. These values have been



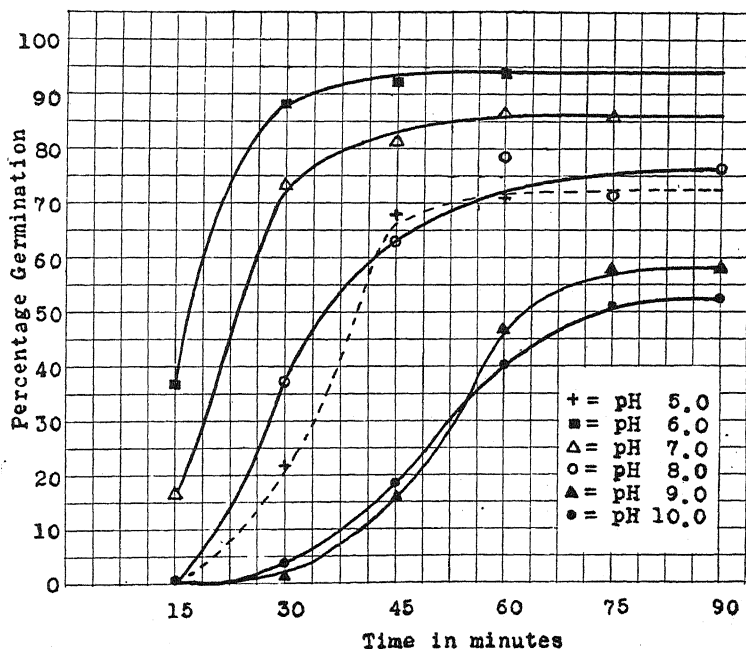
TEXT FIG. 3. The percentage germination and amount of pollen-tube growth in the cultures of the gelatin series. The growing pollen tube is appreciably more sensitive to changes in pH than is the pollen grain.

TABLE 6. *Rate of Germination of Sweet-pea Pollen in Media of Varying pH*

Time in Minutes	Percentage Germination					
	Check	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 10.0
15.....	24.2	36.6	16.5	0	0	0
30.....	81.9	88.2	74.2	36.6	2.0	3.8
45.....	88.6	92.9	80.9	63.4	15.9	18.0
60.....	89.4	93.9	86.9	78.3	46.5	40.0
75.....	—	—	86.4	71.4	58.9	51.0
90.....	—	—	—	75.9	58.2	52.6

plotted in text figure 4. The cultures were incubated at 26° C., and the counts were made at 15-minute intervals as rapidly as possible at room temperature. The order in which the different media stand in regard to rate of germination corresponds rather closely to the positions they occupy with reference to total germination and growth. A pH 5.0 culture was not included in the particular set discussed here. Some difficulty was experienced in getting an accurate count in media of this pH in the necessarily

limited period of time available, because of the relatively large amount of bursting of the grains and young tubes. It is clear from other tests, however, that the rate of germination at pH 5.0 stands below that for pH 6.0 and pH 7.0.



TEXT FIG. 4. Rate of germination of sweet-pea pollen in media of different hydrogen-ion concentrations. A pH 5.0 culture was not included in the set represented by the solid lines. For comparative purposes, however, the values obtained at this pH in a similar test are given. These values lie along the broken line.

#### DISCUSSION

In determining the relations between hydrogen-ion concentration and the germination and growth of pollen, the effect of any other cation varying simultaneously must, perforce, be taken into consideration. The cations of most neutral salts, when these are added singly, even in small amounts, are so toxic to pollen as to preclude growth. Possibly this situation in the pollen grains of angiosperms is unique, for the writer has been unable to find mention of a comparable case among the much more extensive experiments that have been published on the relation of hydrogen-ion concentration to the germination of spores of several of the fungi. It may be that the fungous spores are relatively resistant to changes in the concentration of sodium and potassium even when these are present in an unbalanced condition; or that other ions present in the nutrient media commonly used may counteract their effects. It has been amply demonstrated, however, that,

in the case of pollen cultivated in highly purified synthetic media, the effect of the base added to regulate the hydrogen-ion concentration is, in itself, very intense and must be controlled. A means of effecting this, in the case of the sweet pea at least, was found in adding  $\text{CaCl}_2$  in suitable concentration to the media. It would carry us too far from our main thesis to discuss here the nature of this phenomenon.

It will have been noted that the lower limit of hydrogen-ion concentration that permitted some germination differed in the phosphate and gelatin series. At pH 3.0 in the former media 19-percent germination was secured, whereas none was obtained in the gelatin series at either pH 3.0 or pH 4.0. We are of the opinion that the results from the gelatin series afford a more accurate picture of the true relations. In view of the low concentration of the phosphates and of their very weak buffer effect from pH 3.0 to pH 6.0, it appears probable that the initial hydrogen-ion concentrations of the cultures in the phosphate series were not maintained. The extent to which the pH was changed in the presence of the growing tubes is not known, but if a shift in the direction of the neutral point took place in the more acid cultures in the phosphate series the observed difference, in large part at least, would be accounted for. The results in the two series as they stand do, however, show a rough agreement in that both manifest an increase in amount of germination over their common range as the neutral point is approached. In the second series, the gelatin in the media may have diminished the rate at which water was delivered to the pollen grains. In some forms (Martin, 6; Anthony and Harlan, 1) this has been shown to be an important factor in germination.

The results in the gelatin series indicate that the optimum for germination lies in the vicinity of pH 7.0, the neutrality point. The two separate tests made are not in precise agreement on this point, however; the first gave the highest germination at pH 7.0, but in the second the germination was slightly higher at pH 6.0. Both tests showed decreases at pH 8.0 and pH 5.0. Taken together these experiments indicate that, while an optimum may exist between pH 6.0 and pH 7.0, the zone of hydrogen-ion concentration that will permit high germination is not rigidly defined. There is probably a range of 2.5 pH in the vicinity of the neutral point where germination is not greatly affected by changes in acidity.

The range of hydrogen-ion concentration that will permit strong growth is significantly narrower than that allowing a high percentage of germination. In the phosphate series the greatest amount of growth occurred at pH 6.2. Greater reliance, perhaps, is to be placed upon the results obtained in the gelatin series. Here the optimum growth occurred at pH 6.0; at pH 5.0 the amount of growth was 47 percent less, and at pH 7.0 it decreased 26 percent. A further sharp decline occurred at pH 8.0, where the amount of growth was about one third of that obtained at the optimum.

These results show that the favorable range of hydrogen-ion con-

centration for pollen-tube growth is relatively narrow. A more detailed investigation may reveal that the optima for germination and growth do not differ greatly. It appears fairly certain, however, that germination is affected less profoundly than growth by deviations from its optimum. On the basis of general considerations, it might have been predicted that the conditions for germination of pollen would be found to be less exacting than those for the subsequent growth of the tubes. In nature, germination occurs in an external environment subject to relatively great vicissitudes, whereas the growing pollen tubes are highly active protoplasts adapted to the presumably quite constant internal environment of the pistil.

The mode by which hydrogen-ion concentration affects the pollen-tube growth is largely a matter of speculation. It has previously been shown by Brink (3) that the time relations of the growth process simulate those of an autocatalytic reaction. It has been demonstrated also that elongation of the tubes in artificial media is related to the digestion of the reserve food materials contributed by the pollen grain. In the case of the sweet pea these stored substances are largely fats, and their hydrolysis may constitute the most important chemical reaction in growth. If, as seems not improbable, the other reactions involved wait upon this one, then it is the "master reaction" according to Robertson's (7) hypothesis. If this conception really applies to the case in hand as outlined, the effect of the concentration of hydrogen ions on growth may be a very direct one. It is known that the action of the fat-splitting enzyme lipase is favored by a certain amount of free acid. The maximum rate of germination of the pollen and the greatest amount of growth occur at pH 6.0. Perhaps this is due in large part to the immediate effect of this concentration of hydrogen ions upon the digestion of the reserve food. There are other possibilities which, perhaps, can not be discussed with profit at this time.

#### SUMMARY

1. A method is described whereby the effect of the hydrogen-ion concentration on pollen germination and pollen-tube growth in a synthetic medium may be determined. This method involves the control of the very striking toxic effect of such cations as K and Na introduced into the cultures in adjusting the pH.

2. The highest percentage of germination was secured at pH 7.0, although the values obtained at pH 6.0 and pH 8.0 are not much lower. It seems probable that, while it lies between these limits, the optimum hydrogen-ion concentration for germination is not sharply defined.

3. The zone of hydrogen-ion concentration favorable for pollen-tube growth is relatively narrow. The optimum is in the vicinity of pH 6.0. Above and below this point, growth falls off rapidly.

4. It is pointed out that the difference in tolerance to pH of germination

and of the subsequent development of the tube is in accord with the relative variability in the conditions under which these phenomena occur in nature.

5. The suggestion is offered that the hydrogen-ion concentration may modify pollen-tube growth through a direct effect upon the chemical reactions attending the digestion of the reserve food materials.

6. It is shown that, even when the known variables are carefully controlled, fluctuations in germination and growth of pollen are still so great that large numbers must be used and frequent repetitions made in order to get an adequate statistical representation of the facts.

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# STUDIES IN WOOD DECAY V. PHYSIOLOGICAL SPECIALIZATION IN *FOMES PINICOLA* FR.

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## INTRODUCTION

The idea of physiological specialization in the fungi has undergone extensive development during recent years. In fact, a considerable amount of literature on this subject has appeared since about 1890, and it has been demonstrated beyond all reasonable doubt that physiological specialization exists in a great number of fungi belonging to widely separated families. The rusts have received particular attention in regard to this point, and many interesting as well as important facts have been brought to light by the work of Eriksson,<sup>1,2</sup> Dietel,<sup>3</sup> Stakman,<sup>4</sup> Hungerford,<sup>5</sup> and others.

Other genera of fungi, as, for example, *Erysiphe*, *Glomerella*, *Sphaeropsis*, *Rhizoctonia*, *Septoria*, and many others, have also been investigated, but as far as the writer is able to ascertain little has been done along the line of physiological specialization in the case of wood-destroying fungi. It is true that some work has been done on these forms, but the emphasis has been on the morphological rather than on the physiological aspects of the question.

In their general life history, many of the wood-destroying fungi differ from most other fungi in that they may, as a result of a single infection, inhabit a single host plant for a great number of years. For example, Boyce<sup>6</sup> has determined that *Polyporus amarus* may vegetate in the trunk of incense cedar (*Libocedrus decurrens*) for a period of 300 years. If then the character and general properties of the host plant exert any influences on the fungi infecting it tending to produce physiological specialization, strains, or varieties, it would not seem unreasonable to suppose that the chances would be very good for the production of such specialized forms or varieties among the wood-destroying basidiomycetes.

<sup>1</sup> Eriksson, J. Ueber die Specialisierung des Parasitismus bei den Getreiderostpilzen. Ber. Deutsch Bot. Ges. 12: 292-331. 1894.

<sup>2</sup> Eriksson, J. Ueber die Specialisierung des Getreideschwartzrostes in Schweden und anderen Länden. Centralbl. Bakt. II, 9: 654-658. 1902.

<sup>3</sup> Dietel, P. Waren die Rostpilze in früheren Zeiten plurivor? Bot. Centralbl. 79: 81-113. 1899.

<sup>4</sup> Stakman, E. C. A study in cereal rusts; physiological races. Minn. Agr. Exp. Sta. Bull. 138: 1-56. Pls. 1-9. 1914.

<sup>5</sup> Hungerford, C. W., and Owens, C. E. Specialized varieties of *Puccinia glumarum* and hosts for variety *tritici*. Jour. Agr. Res. 25: 363-401. Pls. 1-6. 1923.

<sup>6</sup> Boyce, J. S. The dry rot of incense cedar. U. S. Dept. Agr. Bur. Plant Ind. Bull. 871: 1-58. 1920.



There is also a very important practical aspect to this question. It is usually considered very poor practice, from the standpoint of forest sanitation, to allow infected trees to remain standing on an area upon which it is expected to raise future forest crops even though these infected trees are of a different genus from those it is expected to grow eventually. A concrete case will serve to elucidate this point. The white pine stands of the Inland Empire contain a considerable number of inferior species such as white fir and hemlock. These latter species are often heavily infected with heartrot, and many of such infected trees remain standing on the area after logging operations are completed. This is particularly true if broadcast burning does not follow the logging operations. These infected trees remain on the area at least during the early growth of the second crop. Very often the same species of fungi which cause the heartrots in these remaining trees also cause heartrots in the trees with which it is hoped to restock the area. Thus, *Trametes pini* causes more or less similar heartrots in fir, spruce, larch, and pine. *Fomes pinicola* causes a red-brown sapwood rot in spruce, larch, fir, pine, and hemlock. *Echinodontium tinctorium* causes a heartrot in practically all the western true firs, Engelmann spruce, Douglas fir, and western hemlock. Many other examples might be given, but these are sufficient to illustrate the point. It is generally assumed, therefore, that these infected trees, remaining on the area after logging, constitute a menace to the future forest crop even though it may be of a different genus of trees. If, on the other hand, physiological specialization has developed to the extent that the strain common to white fir is limited to white fir, the Douglas fir strain is limited to Douglas fir, etc., it is evident that the expense of felling and destroying these infected remaining trees might be eliminated. The writer volunteers no expression of opinion on this point at this time, but merely calls attention to such a limiting factor in cross-infection.

It must be obvious that artificial infection experiments with the heartrot fungi are especially difficult and that it would take many years before any reliable data could be obtained. However, studies can be made on the physiological characteristics of these forms which may, at least, indicate the desirability of undertaking such artificial infection experiments no matter how difficult or time-consuming the work may be.

The present paper reports the results obtained from a physiological study of four cultures of *Fomes pinicola* Fr. obtained from four different hosts, namely, Douglas fir (*Pseudotsuga taxifolia*), white fir (*Abies grandis*), western hemlock (*Tsuga heterophylla*), and western white pine (*Pinus monticola*).

It is recognized that, from the standpoint of practical application, a more important wood-destroying fungus might have been chosen. *Fomes pinicola* occurs much less frequently in living trees than it does in dead and down timber, and is a sapwood rot rather than a heartwood rot. On the other hand, it is a very rapidly growing form, can be grown to some extent

in liquid media, and in general lends itself admirably to cultural work. It is also recognized that the results here recorded would have far greater value if duplicate cultures of each strain might have been obtained and had been compared with each other.

#### SOURCE OF CULTURES

The four strains of *Fomes pinicola* studied were all obtained from sporophores by the tissue method. The trees from which the sporophores were collected were dead but still standing. In each case there was some evidence that the trees had not been dead long and that they probably had become infected while still in the living condition. The belief that the trees had not been dead for any great length of time was based on the fact that they still bore their smaller branches.

The sporophores obtained on Douglas fir, western hemlock, and western white pine were collected in the vicinity of Metaline Falls, Washington, September 4, 1922. The sporophore obtained on white fir was collected near Moscow, Idaho, September 23, 1922. The general climatic conditions of these two localities are very similar, making it improbable that any variations observed in the different strains were due to past geographic influences.

Within a few days after the date of collection of the sporophores, cultures were made from them. In all cases Thaxter's hard potato agar served as the culture medium. All cultures of the various strains in any particular phase of the work had exactly the same cultural history not only with reference to the culture medium employed but also with reference to the number of sub-cultures that had been made. For the sake of brevity the various strains are designated as follows:

Douglas fir strain.....	D. F.
White fir strain.....	W. F.
Hemlock strain.....	H.
Western white pine strain.....	W. P.

#### METHODS OF GROWING THE MYCELIUM AND OF OBTAINING THE CARROT EXTRACTS AND FUNGUS MEALS

Some studies on enzym activity in the wood-destroying fungi have already been made in this laboratory. In this work the same methods are employed as in the former studies.<sup>7, 8</sup> Sliced carrots were placed in Erlenmeyer flasks of 2,000-, 1,000-, and 500-cc. capacity. No water was added to the flasks, since it was found that the carrots contained sufficient moisture for the growth of the fungi. The same amounts of sliced carrots were placed

<sup>7</sup> Schmitz, H. Enzyme action in *Echinodontium tinctorium* Ellis and Everhardt, Jour. Gen. Physiol. 2: 613-616. 1919.

<sup>8</sup> Schmitz, H. Enzyme action in *Polyporus volvatus* Peck and *Fomes ignarius* (L.) Gillet. Jour. Gen. Physiol. 3: 795-800. 1921.

in all the flasks of the same capacity, and two flasks of each capacity were inoculated with each of the four different strains.

All the cultures were incubated at 28° C., and, while still in an actively growing condition, the contents of the flasks were removed, the fungous mats were separated from the carrot, washed with distilled water, and then dried. Since *Fomes pinicola* develops a very definite mat, this separation offered no particular difficulty. The fungous mats were quickly dried by a current of warm air, then finely ground, and the resulting meal was dried to constant weight over sulfuric acid in a desiccator. This fungous meal was subsequently employed in the study of the intracellular enzym activity of the various strains.

The carrots and liquid remaining after the removal of the fungous mats were placed in cheesecloth sacks and the juice was extracted. This was later filtered and preserved by the addition of 2 percent toluol. The carrot extract was subsequently used for the study of the extracellular enzym activity of the various strains.

#### GROWTH CHARACTERISTICS ON CARROT MEDIA

The character of the growth of the various strains of *Fomes pinicola* differed quite markedly on the carrot culture medium. In the case of the W. F. strain, the mat was much heavier and more coriaceous than in the case of any of the other three strains. The Douglas fir mat was quite fluffy in appearance and exhibited no apparent tendency to become coriaceous. As indicated in table 1, the final yield of fungous meal also varied considerably. Since not all the fungous tissue was recovered in the process of separating the mats from the culture medium, and since there was a little loss in grinding, these figures do not represent the absolute amount of growth in each case; but they are sufficiently accurate to serve as an indication of the relative amount of growth.

TABLE 1. *Approximate Yield of Fungous Meal Obtained From Four Strains of Fomes pinicola*

Source of Culture	Yield of Meal in Grams
Douglas fir. ....	10.2
White fir. ....	67.9
Western hemlock. ....	35.0
Western white pine. ....	24.7

Sugar analyses of the extracts show that the different strains also differed considerably in the utilization of the sugars present in the culture medium.

The relative amounts of sugar remaining in the carrot extracts after the growth of the various strains are shown in table 2. The results given are the averages of two titrations and are expressed as the number of cubic centimeters of 0.05 N potassium permanganate solution required to oxidize the dissolved copper. One cubic centimeter of the extract and 20 cc. of standard Fehling's solution were used in each case.

TABLE 2. *Sugar Remaining in the Carrot Extracts After the Growth of Four Strains of Fomes pinicola*

Source of Culture	0.05 N KMnO <sub>4</sub> (cc.)
Douglas fir.....	29.20
White fir.....	8.45
Western hemlock.....	13.35
Western white pine.....	27.75

The data here presented are sufficient to indicate that the growth of the four strains differs to a quite marked degree. It is evident that the amount of sugar remaining in the carrot extract is in inverse proportion to the amount of dry fungous tissue produced.

The carrot extracts after the growth of the different strains had a similar hydrogen-ion concentration in each case, namely, pH 2.8. The titratable acidity, however, varied considerably, as indicated in table 3.

TABLE 3. *Titratable Acidity of the Carrot Extracts After the Growth of Four Strains of Fomes pinicola*

Source of Culture	0.02 N NaOH Required to Neutralize 1 cc. of Extract	pH
Douglas fir.....	8.00	2.8
White fir.....	4.90	2.8
Hemlock.....	6.25	2.8
Western white pine.....	5.00	2.8
Culture medium (not inoculated).....	0.60	5.4

This apparent discrepancy between the titratable acidity and the pH can undoubtedly be explained on the basis of a different buffer index in the various extracts. The four strains of *Fomes pinicola* are, therefore, similar in their effect on the shifting of the hydrogen-ion concentration of the substrate when carrots serve as the culture medium.

#### EXTRACELLULAR ENZYM ACTIVITY OF THE DIFFERENT STRAINS AS INDICATED BY THE ENZYM ACTIVITY OF THE CARROT EXTRACTS

##### Esterases

The esterase activity of the different extracts was determined by the action of the extracts on 1-percent solutions of methyl acetate, ethyl formate, ethyl acetate, triacetin, and olive oil emulsion. In all cases the incubation period was twenty-one days. After the incubation period hydrogen-ion-concentration determinations were made of the enzym cultures, but the data thus obtained gave results of questionable value, in consequence, no doubt, of the fact that on boiling the extracts to be used in the control cultures a copious precipitate was thrown down, thus appreciably affecting the buffer index in these cultures. For this reason titration of the cultures, after the incubation period, with N/50 NaOH was resorted to. It

was, however, first determined that the short boiling of the extracts did not appreciably change the titratable acidity of the extracts. The details of the titrations were to filter the enzym cultures, adding one cc. of the filtrate to 50 cc. of distilled water and using methyl red as an indicator. All titrations were made in duplicate, and the results show that esterase activity was not very pronounced in any of the carrot extracts. Esterase activity when methyl acetate or ethyl acetate is used as a substrate is shown only in the cases of the W. F. and W. P. strains; all strains show apparent esterase activity when triacetin is used as the substrate; and the hemlock strain shows slight esterase activity when olive oil emulsion is employed as the substrate. None of the strains shows apparent esterase activity when ethyl formate is used as the substrate.

The results, although far from conclusive, show some similarity in the W. F. and W. P. strains with reference to esterase activity.

### Carbohydases

The action of carbohydases in the various extracts was determined by the action of the extracts on 1-percent solutions of maltose, lactose, sucrose, raffinose, potato starch, inulin, white fir cellulose, and hemicellulose from date-seed endosperms. It was first found necessary to determine the effect of boiling on the extracts with reference to the total amount of reducible sugars present. In the enzym studies previously published by this laboratory, the customary procedure employed to "kill" the enzymes in the control cultures was to autoclave them for ten minutes at ten pounds' pressure. When this method was used to inactivate the enzymes present in the extracts, it was found that appreciable increases in the amounts of reducible sugars were produced. This was, no doubt, due to a hydrolytic effect of the acids in the extracts on the sucrose present. Preliminary experiments showed that 5-cc. amounts in ordinary non-sol test tubes could be plunged in boiling water for at least two minutes without increasing the amount of reducible sugar present, and this method was therefore used to inactivate the enzymes. Even with this treatment a quite copious flocculent precipitate was thrown down. In all cases 5 cc. of the extract were added to 5 cc. of the sugar solutions, and all cultures were set up in duplicate. One cc. of toluol was invariably used as a preservative. After varying periods of incubation the cultures were filtered, and 1-cc. samples were treated with 20 cc. of Fehling's solution.

Considerable variation in carbohydase activity is exhibited in the different extracts. When inulin is employed as a substrate, appreciable activity is indicated only in the case of the D. F. strain. On the whole the data obtained do not indicate any constant outstanding variations in extracellular carbohydase activity in the different strains of *Fomes pinicola*.

### Glucosidases

The presence of glucosidases in the carrot extracts was determined by the action of the extracts on a 1-percent solution of salicin and amygdalin. After five-day incubation periods the cultures were filtered, and sugar determinations were made of 1-cc. samples of the filtrates.

Glucosidase action with reference to its action on both salicin and amygdalin is greatest in the W. P. extract. The D. F. strain extract shows the minimum amount of action on salicin. When amygdalin served as the substrate, with the exception of the pronounced action produced by the W. P. strain extract already noted, the action of the extracts of the various strains is very similar.

### Tannase

The tannic acid cultures were incubated twenty days. After the incubation period the cultures were filtered, and to 5 cc. of the filtrate, 5 cc. of a 1-percent albumin solution were added. After standing a short interval, an excess of sodium chlorid was added and the cultures were again filtered. Two cc. of the second filtrate were then titrated against N/100 iodine solution according to the method suggested by Jean.<sup>9</sup> The titration was carried on very slowly, but even when so carried on the end point was not definite in that after standing several hours all traces of free iodine disappeared. This disappearance may be due to the fact that the carrot extracts were very acid and undoubtedly contained traces of a number of organic acids which interfere with the titration. The results of these titrations, therefore, are considered to be of little or no value in so far as determining either the presence or the absence of tannase in the extracts is concerned.

### Urease and Amidase

For this phase of the work 0.5-percent solutions of urea and acetamid were prepared. Five-cc. samples of the extracts were added to 50-cc. samples of the solutions in ordinary wash bottles and incubated for a period of seven days. At first the hydrogen-ion method suggested by Schmitz and Zeller was employed, but it soon became apparent that, since the various carrot extracts were quite acid in reaction, any ammonia which might have been liberated through urease or amidase action would immediately combine with the acids contained in the extracts and could therefore not be demonstrated by aspirating the cultures and subsequently passing the air current through distilled water to which an indicator had been added. For this reason the cultures, after the incubation period, were filtered and 1-cc. samples of the filtrate were added to 50 cc. of distilled water. These solutions were then titrated with N/50 NaOH with methyl red as an indicator. The results of these titrations were negative in each case.

<sup>9</sup> Jean, F. Die Bestimmung des Tannins und der Gallussäure. *Ann. Chim. Anal. Appl.* 5: 134-140. 1900.

### Catalase

The presence of this enzym in the extracts was demonstrated by the action of the extracts upon a 3-percent solution of hydrogen peroxid. When 5 cc. of the extract were added to 5 cc. of peroxid a slow liberation of oxygen resulted in all cases, but it was in such limited amounts as to be of no indicative value with respect to showing any differences in the various extracts.

### Rennet

When 5 cc. of the carrot extracts were added to 10 cc. of fresh milk, coagulation occurred immediately. The milk also coagulated when 5 cc. of the boiled extracts were added to the milk. Coagulation was no doubt due to the acidity of the extracts.

### Protease

Tryptic and ereptic fermentation were studied by the use of albumin, peptone, casein, and fibrin in 1-percent solutions. Five cc. of the extracts were in all cases added to 5 cc. of the solutions. The biuret test was employed in testing for the action of erepsin, and the tryptophan test in testing for tryptic fermentation. Tryptic fermentation was demonstrated only in the cases of the D. F., W. F., and W. P. extracts when albumin was used as a substrate, but negative results were obtained in the case of the H. extract with the same substrate. The control in all cases gave negative results.

### INTRACELLULAR ENZYM ACTIVITY

In this phase of the work the ground-up mycelium was employed. The preparation of the mycelial meal has been previously described in this paper. In all cases, unless otherwise stated, 0.1 gram of mycelial meal was added to 10.0 cc. of substrate solution plus 1.0 cc. of distilled water. In the control cultures the enzymes were inactivated by adding 1.0 cc. distilled water to the fungous meal and immersing the test tubes in boiling water for five minutes. In general, the methods of analysis are identical with those employed in the study of the extracellular enzym activity.

### Esterases

The results of the study of the esterase activity of the fungous meal showed that positive esterase action is evident when methyl acetate, ethyl formate, and triacetin serve as substrates. In all three cases there appears to be considerable similarity between the D. F. and W. P. strains and between the W. F. and H. strains.

### Carbohydases

In this phase of the work, the method of procedure was identical with that used in the study of the extracellular carbohydases except that in this (the

latter) phase of the work 2.0 cc. of the filtrates were used for the sugar determination.

Positive results were obtained with each carbohydrate solution. The amount of hydrolysis in the case of sucrose, raffinose, and inulin, however, is quite limited. No general statement can be made regarding the activity of any one strain on all the substrates. When some substrates were used one strain would show the greatest activity, while, when other substrates were employed, an entirely different strain would show the greatest activity.

### Glucosidase

The action of the fungous meal upon 1-percent salicin and amygdalin seemed to indicate some similarity in the cases of the D. F. and W. P. strains. These two strains are much more active in this respect than are the W. F. and H. strains.

### Tannase

The results of the study of the tannase activity of the fungous meal of the different strains showed that tannase action was very limited in all four strains.

### Urease and Amidase

In order to determine the presence or absence of amidase and urease in the fungous meals, acetamid and urea were used as substrates and the hydrogen-ion method previously mentioned in connection with the determination of the presence of these enzymes was employed. The results in all cases were negative.

### Proteases

In this phase of the work the same methods and substrates were employed as in the determination of extracellular protease activity. Here also the results were entirely negative.

### MIXED CULTURES

When plates of nutrient media are inoculated with two or more fungi, various effects may be produced. Thus, Zeller and Schmitz,<sup>10</sup> working with the wood-destroying forms, showed that under some conditions the hyphae resulting from the two inoculations may mix without any apparent influence. This condition usually results when the two inoculations are made with the same fungus. In other cases an inhibition of growth may result, and in still other cases the growth of the organisms may be stimulated.

In order to determine the influence of the different strains of *Fomes pinicola* on each other, agar plates were prepared. The nutrient agar had the following composition:

<sup>10</sup> Zeller, S. M., and Schmitz, H. Studies in the physiology of the fungi VIII. Mixed cultures. *Ann. Mo. Bot. Gard.* 6: 183-192. 1919.



Extract from 200 grams potato.	
Cane sugar.....	20.0 grams
Potassium nitrate.....	10.0 grams
Monobasic potassium phosphate.....	5.0 grams
Agar.....	20.0 grams
Distilled water to make.....	1,000.0 cc.

These plates were inoculated so that all possible combinations of the strains would result. The following combinations were prepared in duplicate and incubated at 28° C.

1. D. F.....	D. F.
2. D. F.....	W. F.
3. D. F.....	H.
4. D. F.....	W. P.
5. W. F.....	W. F.
6. W. F.....	H.
7. W. F.....	W. P.
8. H.....	W. P.
9. W. P.....	W. P.
10. H.....	H.
11. D. F.-W. F.....	H.-W. P.

Plates VIII and IX show the growth conditions on these plates after twenty-one days. The plates were, however, kept under observation for sixty days.

The effects of the various strains upon each other can be seen in Plates VIII and IX. In all plates having two inoculations of the same strain there seemed to be no inhibiting or stimulating effect. The colonies in these cases grew into each other, eventually completely covering the surface of the plates just as if the growth had resulted from a single inoculation. On the other hand, whenever the plates were inoculated with two different strains the colonies did not intermingle. In these cases a clear line of demarcation remained between the two colonies.

#### NITROGEN RELATIONS

The availability to fungi of various nitrogenous substances can probably be studied to the best advantage when liquid culture media are employed. Because, however, of the poor growth in liquid media of many of the wood-destroying forms, solid culture media were employed in which the source of nitrogen was varied.

A stock agar was prepared having a composition as follows:

Agar.....	20.00 grams
Dextrose.....	20.00 grams
Magnesium sulfate.....	.50 gram
Monobasic potassium phosphate.....	1.00 gram
Distilled water to make.....	1,000.00 cc.

While still hot and in a liquid state, 200-cc. amounts of this culture medium were pipetted into small Erlenmeyer flasks. To these subsequently were added the following substances: casein, albumin (egg), peptone, potassium nitrate, sodium nitrite, ammonium nitrate, ammonium sulfate, and urea. No nitrogenous substance was added to one flask, and the contents of this flask served as a control.

The flasks and contents were then sterilized, and following sterilization the contents were plated in the usual manner. For each *Fomes pinicola* strain two plates were prepared of each culture medium.

After cooling, the plates were inoculated with the different strains by means of small squares of the respective fungus cut from plates in which potato agar served as a culture medium. It is clearly recognized that some form of nitrogen is introduced to the test plates by these inocula. The growth of the fungus in the nitrogen-free plates is further evidence of this fact. After inoculation the plates were incubated at 28° C., and the diameter of the colonies was measured after six, ten, and sixteen days. The diameters were measured in two directions, and the diameters shown in table 4 are the averages of duplicate plates. The diameter of the plates was approximately 100 mm.

TABLE 4. *Effect of Various Nitrogenous Substances on the Rate of Growth of Four Strains of Fomes pinicola*

Source of Nitrogen	After No. Days Indicated	Diameters of Colonies in Centimeters			
		D.F. Strain	W.F. Strain	H. Strain	W.P. Strain
Casein.....	6	52	65	48	56
	10	90	90	74	86
	16	100	100	100	100
Albumin.....	6	17	38	19	16
	10	25	65	27	19
	16	61	72	37	23
Peptone.....	6	46	60	50	42
	10	80	90	86	80
	16	100	100	100	100
Potassium Nitrate.....	6	42	54	46	38
	10	78	87	73	80
	16	100	100	100	100
Sodium Nitrite.....	6	No growth	No growth	No growth	No growth
	10	" "	" "	" "	" "
	16	" "	" "	" "	" "
Ammonium Nitrate.....	6	51	59	60	50
	10	85	90	87	72
	16	100	100	100	100
Ammonium Sulfate.....	6	45	56	47	42
	10	76	85	80	72
	16	100	100	100	100
Urea.....	6	No growth	No growth	No growth	No growth
	10	" "	12	10	" "
	16	" "	38	25	17
Control.....	6	44	54	43	47
	10	77	90	88	85
	16	100	100	100	100

In general, the W. F. strain made the greatest and heaviest growth. All forms made the heaviest growth on the peptone plates, about equal growth on the casein and ammonium nitrate plates, followed by the ammonium sulfate and potassium nitrate plates in the order named.

On the albumin plates growth was comparatively poor for all strains, but the D. F. and W. F. strains made far better growth than the H. and W. P. strains. When urea served as the source of nitrogen, growth of the D. F. strain was entirely inhibited, while the W. F., H., and W. P. strains grew in amount in the order named.

Practically as good a rate of growth was made on the control plates as on any of the others. This was undoubtedly because sufficient nitrogen was introduced into the plates by the inoculum. The fungous mats were rather thin and delicate, however, although the rate of growth was quite rapid.

These data tend to indicate that the D. F. strain differs from the other strains in regard to its reaction toward urea. The W. F. strain differs from the others in that its growth was usually more rapid and much greater in amount, tending to become more or less fluffy. The H. and W. P. strains, although not exactly similar in appearance, are quite nearly so under the conditions of growth obtaining in these experiments.

Some of the above-noted points are illustrated in Plate X.

#### GROWTH ON LIQUID MEDIA

It is a well known fact that many wood-destroying fungi make little or no growth on liquid culture media. In order to determine whether the four strains of *Fomes pinicola* being dealt with differed with respect to the amount of growth made on liquid media, Czapek's and Richards' solutions were prepared. Each of these solutions was employed in three modifications. These modifications consisted in the substitution of the dibasic phosphate for the monobasic phosphate in one case and in substituting the tribasic phosphate for the monobasic phosphate in the other.

It was intended to make hydrogen-ion determinations of these solutions before and after growth of the fungus in order to determine the amount of change in hydrogen-ion concentration of the solutions brought about by the growth of the various strains of the fungus. However, growth was so slow in all cases that the incubation period had to be extended to fifty days in order to get any appreciable amount of growth. During this period the culture solutions evaporated to approximately one half of their former volume, and for this reason it was felt that hydrogen-ion concentration determinations of the culture solutions would be of little value.

Twenty-five-cc. amounts of the various culture solutions were pipetted into 125-cc. Erlenmeyer flasks. Duplicate flasks were prepared of each solution for each strain. After sterilization in the usual manner, the culture flasks were inoculated from plate cultures of the various strains, using very small inocula. The entire series was incubated at 28° C. for fifty days before the final observations were taken.

Since the growth was very limited in all cases, the fungous mats were not dried and weighed. In general, the best growth was made by the H. and D. F. strains, in the order named.

The H. strain made the best growth on the mono- and dibasic Richards' solution; the D. F. strain made its best growth on the di- and tribasic Richards' solution, while only limited growth was made on the monobasic solution.

Only in the cases of the H. and D. F. strains was any apparent growth made on Czapek's solution and then on the monobasic solution only. The W. P. and W. F. strains made only very limited growth on di- and tribasic Richards' solution and no apparent growth on the monobasic solution. The very limited growth made by the W. F. strain in liquid culture media is a little surprising since this strain in general makes the heaviest and most rapid growth on solid culture media.

#### WOOD-DESTROYING PROCLIVITIES OF THE VARIOUS STRAINS

Since a wood-destroying organism is being considered in the present work, the wood-destroying proclivities of the various strains are naturally of great interest. In most of the work that has been done dealing with the decay of wood induced by a given wood-destroying fungus, very little attention has been placed upon the source of the culture, and the question of the possibility of physiological specialization does not seem to have been given the attention to which it would seem to be entitled.

In order to determine whether there is any variation in the ability of the four strains of *Fomes pinicola* considered to decay different woods, a series of decay experiments were prepared employing not only the wood from the four different hosts from which the strains were isolated, but several others, namely: *Pinus ponderosa* (western yellow pine) heartwood, *Pinus ponderosa* (western yellow pine) sapwood, *Pinus monticola* (western white pine) heartwood, *Larix occidentalis* (western larch) heartwood, *Pseudotsuga taxifolia* (Douglas fir) heartwood, *Abies grandis* (white fir), *Tsuga heterophylla* (western hemlock), and *Picea engelmanni* (Engelmann spruce).

Small blocks, approximately  $1 \times 1 \times 2\frac{1}{2}$  inches in size, were prepared. Although there was some variation in the sizes of the blocks when the different kinds of wood were considered, the blocks of one kind of wood were, within reasonable limits, of the same size. Because of this variation in size between the blocks of the different woods, the results recorded should not be taken to indicate the relative durability of the woods in question.

Either five or six blocks of known weight, as the case might be, of one kind of wood were placed in two-quart Mason jars having about one and one half inches of apple-wood sawdust on the bottom of each jar. The sawdust was added in order to keep the moisture conditions more nearly uniform throughout the incubation period. Four jars were prepared for each species of wood, thus allowing one jar for each strain of the fungus.

After sterilizing the culture jars for thirty minutes at fifteen pounds' pressure and allowing them to cool, they were inoculated in the usual manner from plate cultures of the various strains. The entire series was incubated at 28° C. for a period of six months, after which the blocks were removed from the flasks and again dried and weighed. The results obtained from these experiments are recorded in table 5.

TABLE 5. *The Amount of Decay Produced by Four Strains of Fomes pinicola when growing on several Coniferous Woods*

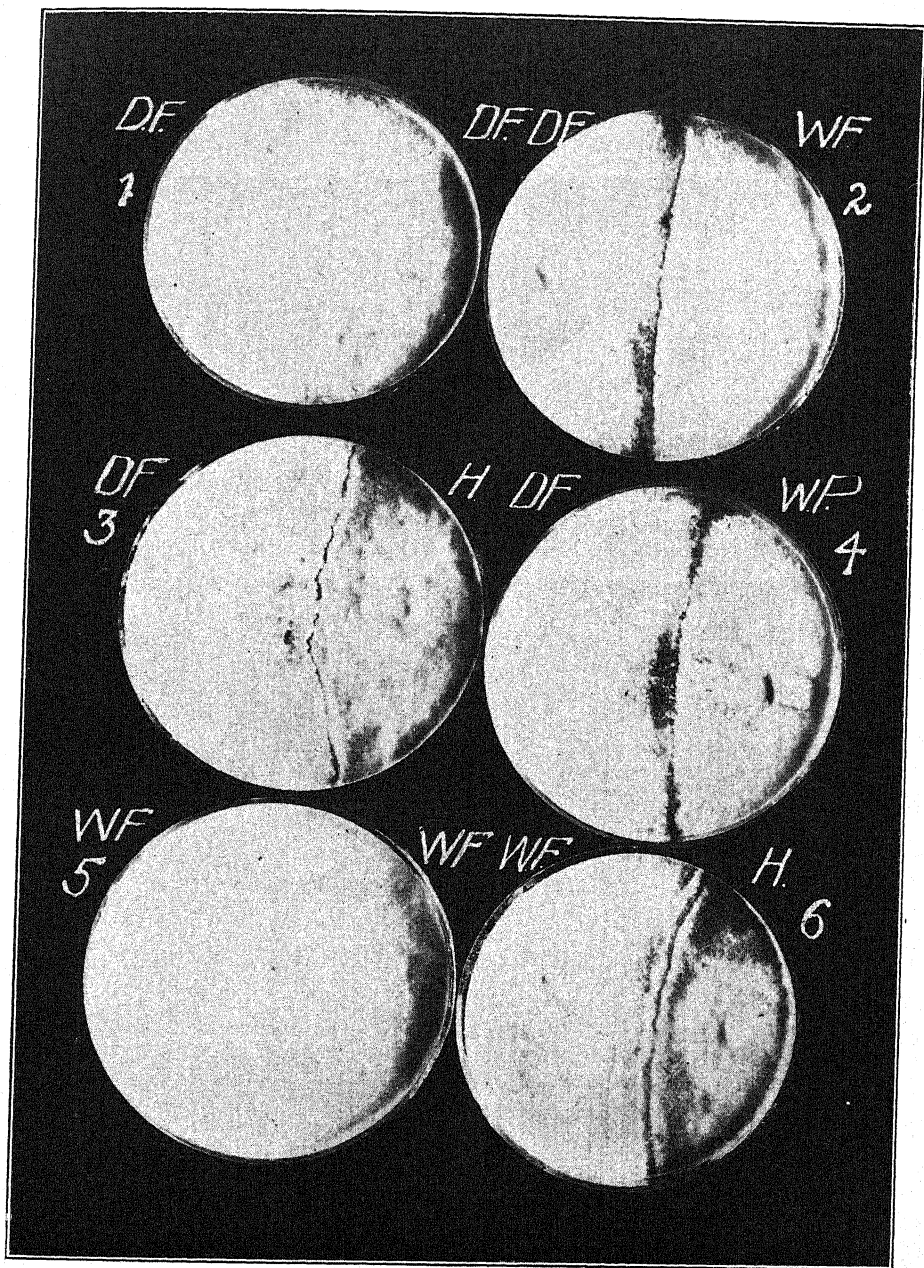
Wood	Strain of Fungus			
	Douglas Fir	White Fir	Hemlock	White Pine
	Loss in Weight, Percent			
<i>Pinus ponderosa</i> (heartwood).....	1.2	5.5	0.8	8.1
<i>Pinus ponderosa</i> (sapwood).....	16.4	2.3	8.6	20.7
<i>Pinus monticola</i> .....	1.1	1.9	1.5	7.7
<i>Larix occidentalis</i> .....	9.6	12.1	14.5	12.2
<i>Pseudotsuga taxifolia</i> .....	5.9	19.8	14.7	4.2
<i>Abies grandis</i> .....	0.2	2.1	0.3	6.5
<i>Tsuga heterophylla</i> .....	12.9	3.8	10.3	10.1
<i>Picea engelmanni</i> .....	10.6	3.6	5.5	5.7
Average all woods.....	7.2	6.4	8.3	9.4

These results are far from conclusive. In only one case is there any indication that the particular strain in question causes a more rapid decay in the wood from which the culture was originally obtained. A glance at table 5 shows that the W. P. strain is the only strain which caused any appreciable decay of white pine heartwood. Attention should also be called to the fact that this particular strain caused the greatest amount of decay in the case of both sapwood and heartwood of western yellow pine. The white pine strain also caused the highest grand average amount of decay, which is a little surprising since, as stated before, the white pine strain is one of the more slowly growing strains. The white fir strain seemed to produce the greatest amount of mycelial tissue on all the wood culture blocks. In some cases the fungous mats of this strain covering the blocks were over one half inch in thickness in the moist condition, and yet this strain caused the lowest grand average amount of decay. The white pine strain, on the other hand, produced only a more or less webby growth over the culture blocks.

The decay of wood under laboratory conditions is unsatisfactory at best, and too much emphasis should not be placed on any results obtained unless those results are based upon an adequate number of tests and can be reproduced time after time.

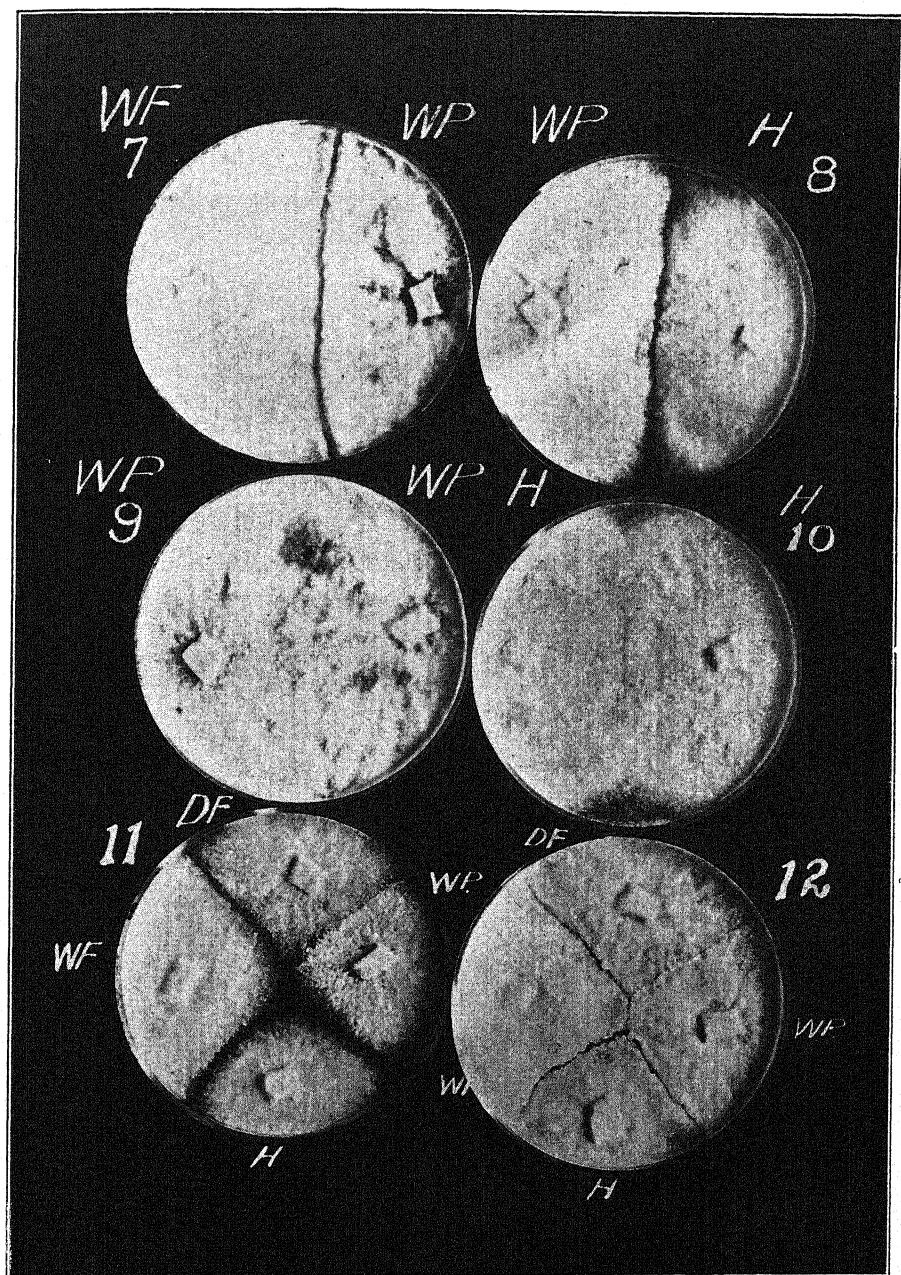
#### SUMMARY AND CONCLUSIONS

Attention has been previously called to the fact that the results of the present investigation would have been of far greater value if duplicate



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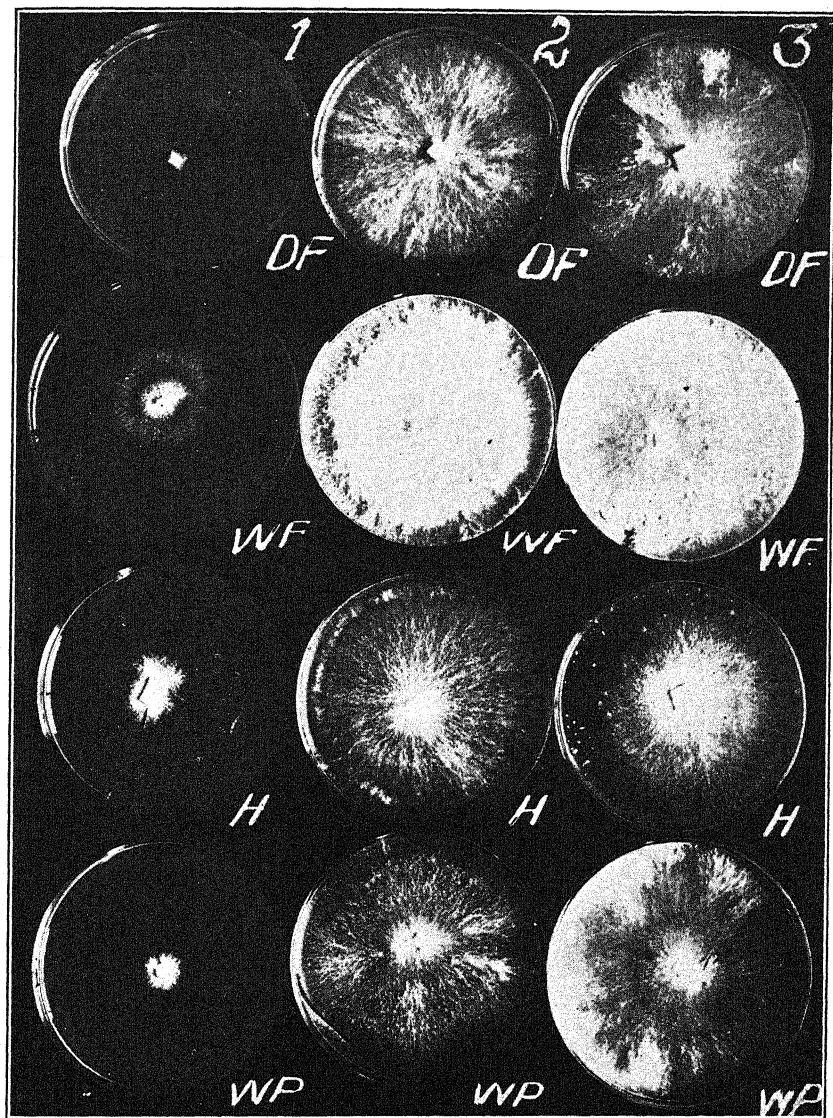




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cultures of the various strains might have been obtained and compared with each other. However, the writer believes that the data presented are sufficiently conclusive to indicate that there may be considerable physiological variation within the species *Fomes pinicola* Fr. Whether or not this variation is the result of host influence is not certain.

The data also indicate the desirability if not the necessity of considering the source of wood-destroying fungous cultures in experiments dealing with the decay of wood under laboratory conditions.

It is also believed that the question of physiological specialization in the wood-destroying fungi is an excellent field for investigation and that many interesting as well as important facts will be brought to light by further work. The results obtained from experiments dealing with the question of physiological specialization in such species of wood-destroying fungi as are found upon both hardwoods and conifers should be particularly interesting, and it is hoped that such a study may be undertaken in the near future.

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SCHOOL OF FORESTRY,  
UNIVERSITY OF IDAHO

#### EXPLANATION OF PLATES

##### PLATE VIII

###### *Mixed Cultures*

- FIG. 1. Douglas fir strain—Douglas fir strain.
- FIG. 2. Douglas fir strain—White fir strain.
- FIG. 3. Douglas fir strain—Hemlock strain.
- FIG. 4. Douglas fir strain—White pine strain.
- FIG. 5. White fir strain—White fir strain.
- FIG. 6. White fir strain—Hemlock strain.

##### PLATE IX

###### *Mixed Cultures (continued)*

- FIG. 7. White fir strain—White pine strain.
- FIG. 8. White pine strain—Hemlock strain.
- FIG. 9. White pine strain—White pine strain.
- FIG. 10. Hemlock strain—Hemlock strain.
- FIG. 11. All strains 16 days after inoculation.
- FIG. 12. All strains 21 days after inoculation.

##### PLATE X

###### *Nitrogen Relations*

- FIG. 1. Douglas fir, white fir, hemlock, and white pine strains on plates with urea as source of nitrogen. Incubation period, 16 days.
- FIG. 2. Same strains on plates with ammonium nitrate as source of nitrogen. Incubation period, 16 days.
- FIG. 3. Same strains on plates with casein as source of nitrogen. Incubation period, 16 days.

## TEMPERATURE AND ANTHESIS

WILLIAM ALBERT SETCHELL

(Received for publication May 14, 1924)

That there are waves of blossoming, or anthesis, in any particular locality and that such waves are more pronounced or less apparent according to the greater or the less amplitude of temperature variation, is a matter of both common experience and scientific knowledge. The seasonal succession of vegetative and reproductive periods, from quiescence during the cold or dry seasons to quiescence again, is an annual occurrence and is repeated in the same sequence and in the same order from year to year with little variation except as the "season" is "early" or "late." The season of vegetative and reproductive activity may be long or short according to the latitude, but even in the moist tropics, on the one hand, and the extreme arctic, on the other, there is to be found at least a slight indication of periodicity, which is usually, if properly observed, greater than might be expected. In the temperate regions, where the winter is cold (typically with ice and snow) and the summer warm (or even hot), the seasonal march from the bursting into leaf and blossom in the spring to the leaf-fall in the autumn occurs in a succession of waves, each accompanied by the anthesis of certain groups. There are a few flowering plants with an extended season of bloom, some even reasonably meriting the title of "perpetual bloomers." There are others which have a short period of general or "full" anthesis, which at certain other times may show scanty flowering or what may be designated as sporadic anthesis. Full anthesis is generally preceded by scattering, often scanty anthesis, resembling sporadic anthesis except that the latter never merges into full anthesis. Full anthesis seems to represent general optimum conditions whereas the scattering anthesis preceding full anthesis presumably represents minimum favorable conditions, while the scattering anthesis lingering after the full anthesis has passed may represent another minimum following the maximum of favorable conditions. On the other hand, sporadic anthesis (I apply the term to scattering anthesis not directly before or after full anthesis but disconnected) may mean a return of favorable conditions for anthesis but for a short period or not fully favorably connected with preliminary or accompanying conditions for flower-bud formation.

For some years my attention has been turned to the relations existing between temperature intervals and geographical distribution in the case of marine algae and marine spermatophytes (*cf.* Setchell, 1893, 1915, 1917, 1920*a*, 1920*b*, 1920*c*, 1922*a*, 1922*b*, 1923, and 1924). The advantage of dealing with aquatic (entirely submerged) plants is that the influence of the one

variable, *viz.*, temperature, may be more readily isolated, observed, and tested. The relations of certain critical temperature points, approximating those 5° C. apart, have been made reasonably certain in the case of aquatics. Connected with the observations on aquatics, particularly of the marine and brackish spermatophytes, has been an attempt to determine in the field their phenologic relations. I may mention here that *Ruppia maritima* has been found to be an aquatic readily grown, and that laboratory cultures have served to emphasize still further the results of field observations. These have been treated in another paper.

While the general temperature rule of the 10°-C. optimal interval for the vegetative and reproductive activity of aquatics seems fairly well demonstrated, the reproductive processes being perfected by the upper interval of 5° C., the question has been raised as to whether this may or may not apply to land plants with a predominance of opinion that it not only may not, but does not. The difficulties in the case of the land plant are many. It exists and carries through its vital processes under a series of variables not easily separable from one another as to their influence, and each series of variables is, in itself, so complex as to defy ready and certain analysis. The ordinary land plant, or more exactly the spermatophyte-geophyte, has, typically at any rate, its roots in the soil and its stems and leaves in the air, while the aquatic may have roots more or less deeply inserted in the mud but its stems and leaves are surrounded by the water. The mud and the water above have an equilibrium, or near it, as to temperature and moisture variables while the water, making all due allowance for diffusion currents, keeps the stems and leaves exposed to a much more nearly constant temperature than can be maintained in the case of the land plant, in respect to which soil temperature, soil moisture, air currents, etc., have each its own effect, and daily or even hourly and seasonal variation in atmospheric temperature and moisture occurs and differs in different portions of both aërial and subterranean organs.

With the desire of obtaining some indication of the behavior of land plants towards the variables of temperature, moisture, and light, I decided to make observations on the associations of some particularly favorable locality where it might be possible to obtain at least closely approximate data on temperature and moisture. The only locality in my vicinity where the conditions were even approximate was the summit of Mt. Tamalpais, across San Francisco Bay from both San Francisco and Berkeley. Near the summit of this mountain, on East Peak, at an altitude of 2375 feet, there has been maintained an observatory of the U. S. Weather Bureau for many years, so that an unbroken series of observations for the years 1898 to June, 1923, have been made and recorded. Unfortunately, since June 1, 1923, these observations have been discontinued. The extreme upper point of East Peak is 2586 feet, so that the station is 200 feet, or a little more, below it. From the situation of the station a nearly level path called the "Twenty

Minute Walk" has been constructed around East Peak at this point. On the south side of the mountain this walk has so nearly the same exposure and conditions of the station as to make the data as closely applicable as might otherwise be possible only with such a multiplication of stations as to include each bush or clump of shrubbery. While the south sector was considered the principal sector on account of its similarity in exposure and meteorological conditions to those of the weather station, the east, northeast, northwest, west, and southwest sectors were always examined in relation to the vegetative and reproductive conditions of their plant cover. Attention was paid in general and chiefly to plants above the path and in its immediate vicinity. A strict record was made at the time of each visit (weekly, fortnightly, or monthly, according to the season) of the vegetative or reproductive activity of each species along the entire path, each according to its sector. Since access to the weather station and the sectors under observation is most convenient by way of the Mt. Tamalpais and Muir Woods Railroad, which winds up the mountain side on its southerly slopes from Mill Valley (altitude about 40 ft.) to the point where the Twenty Minute Walk begins, an excellent opportunity was given of observing from the open cars the behavior of the different species under consideration from the lower levels up and under some variety of exposures. Stops were also made at the "Mesa" (altitude about 1000 ft.) and at "West Point" (altitude 2150 ft.), which seem to be fairly distinct transition places (or near them) in the waves of anthesis as they roll up (spring and early summer) or roll down (late summer and autumn) the southerly slopes of the mountain. These observations served to extend as well as to check the observations near the summit.

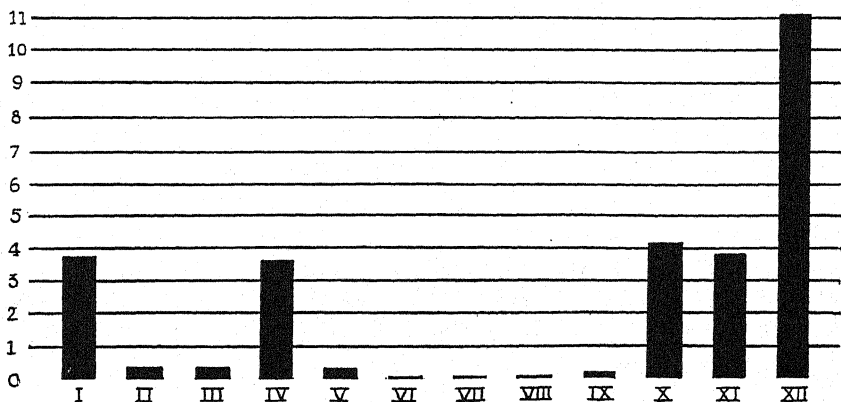
The vegetation of the summit of Mount Tamalpais is of the chaparral type of formation in rocky crevices or stony shallow soil, in consequence of the more or less incomplete disintegration of the San Francisco sandstone which forms the bulk of the surface material of the mountain. Around the Twenty Minute Walk about 70 species were found and of these somewhat less than half were met with (during the year of observations) on the south sector, of which only some 25 species were possible of continuous and undoubted record. With one possible exception, these are all perennials and all indigenous species. Introduced weeds, in spite of favorable situation for introduction, never seem to obtain a firm foothold on the uppermost reaches of Tamalpais. The few exceptions are *Erodium cicutarium*, *Rumex Acetosella*, and *Anychia dichotoma*, which show a single small (and for the last species precarious) colony. The greater number of species (out of the 24 clearly established) on the south sector are shrubs or trees (although not reaching any considerable height on this sector). One only is an annual, two are herbaceous perennials, one is bulbous, while eight are provided with frutescent or suffrutescent bases.

The climate of Mt. Tamalpais has been described by Professor Alexander

G. McAdie in the following paragraph (*cf.* "Summary of the Climatological Data for the United States by Sections," section 14, Central and Southern California):

The climate of the Bay section is unlike that of any other portion of the United States. Owing to the proximity of the ocean the amplitude of the temperature curve is small; but owing to the peculiar surface draughts and the many local circulations, localities only a few miles away from the ocean are sheltered and in these the temperature is both higher and lower than would otherwise be the case. There are no well-marked seasonal changes at San Francisco itself; but at San Rafael, Mount Tamalpais or San Jose, well marked seasonal changes can be noted. The mean annual temperature at San Francisco is about 56°, or practically the temperature of the Pacific ocean in this latitude. The coldest month is January, with a mean temperature of 50°. The warmest period of the year is from the middle of September to the middle of October, when the mean temperature is about 60°. At Mount Tamalpais the mean annual temperature is about 55°, or nearly the same as San Francisco; but the mean temperature for July is 70°, and for January about 40°. During the winter months there is an average vertical gradient of 1° C. fall for every 260 meters elevation; but in summer a marked inversion occurs, and there is a rise in temperature with elevation, at an average rate of 1° C. for every 44 meters. These temperature inversions are due to the water vapor borne through the Gate from the Pacific by the strong west winds. The usual rate of cooling of ascending air is about 1° C. for every 100 meters. On summer afternoons when the surface temperature is about 55° F. (12° C.) a layer of fog about 500 meters in depth spreads over the city. Just above this layer bright sunshine is found, with temperatures ranging from 80° F. to 92° F., or from 27° C. to 33° C.

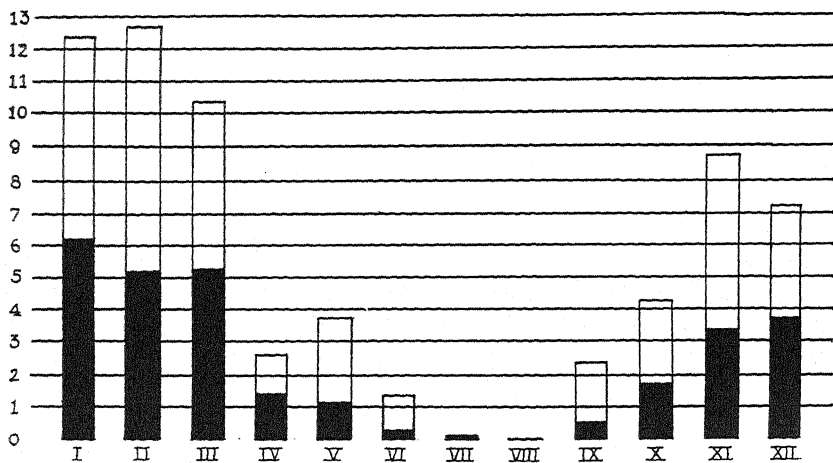
The above statement of McAdie characterizes the climate of Mt. Tamalpais in general as to temperature and its relations particularly to San Francisco. In the same report are tables of temperatures recording the averages of the mean maxima, the means, and the mean minima (in the case of the station on Mt. Tamalpais based on records of 13 years as well as the recorded monthly seasonal, annual, and average amounts of precipitation for the years 1898-99 to 1911-12). I have represented on the graphs (text figures 1-4) the average temperatures and precipitation for the Mt. Tamalpais station as well as those for 1922-1923, the year of my observations.



TEXT FIG. 1. The monthly precipitation at the Mt. Tamalpais Weather Station from June, 1922, to May, 1923; the Arabic numerals representing inches and the Roman numerals designating the months from January (I) to December (XII).



There were five distinguishable waves of anthesis observed on the top of Mt. Tamalpais, *viz.*, one in January and February, one in April and May, one in June, one in July, and one in August and September. Some of these are divisible into two, a greater and a less, but the last is not divisible nor,

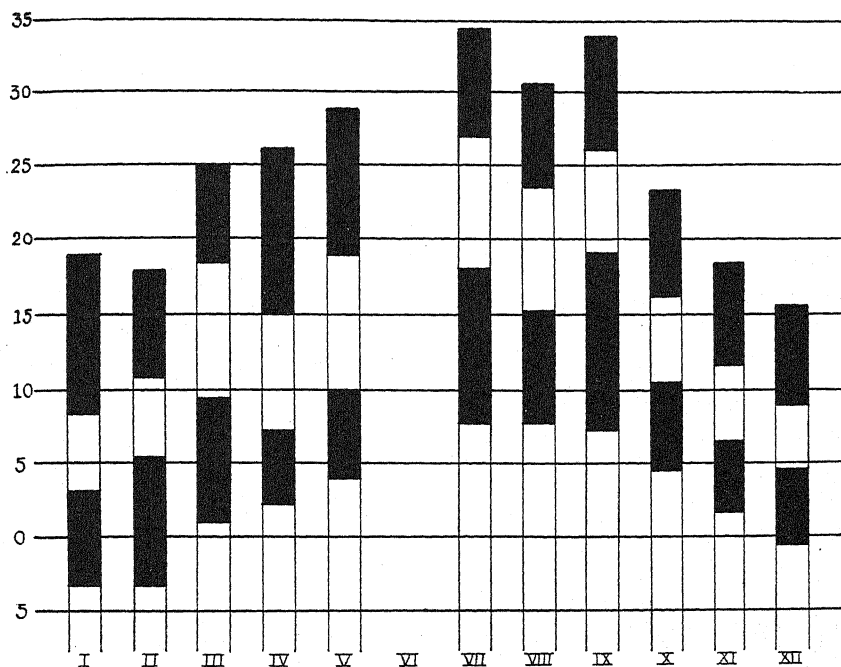


TEXT FIG. 2. The average monthly precipitation, calculated from July, 1898, to June, 1912; symbols as in text figure 1.

as yet, exactly satisfactorily explicable. The first occurs between the mean maxima and mean minima of  $5^{\circ}$  C. and  $10^{\circ}$  C., the second between those of  $10^{\circ}$  C. and  $15^{\circ}$  C., the third between those of  $15^{\circ}$  C. and  $20^{\circ}$  C., the fourth between those of  $20^{\circ}$  C. and  $25^{\circ}$  C., while the fifth probably occurs between those of  $25^{\circ}$  C. and  $30^{\circ}$  C. At least, these are approximately the intervals between the mean maxima and the mean minima of the months in which the successive waves of anthesis occur. The influence of the lowest and of the highest temperatures of each month are not readily to be estimated except as tending to raise the afternoon temperatures and to lower the darkness temperatures after midnight. In some cases their influence may be appreciable, but it seems unsafe to ascribe to them any considerable influence.

The earliest anthesis in the year takes place in January, February, and March. At the top, it begins three to four weeks later than it does well down on the mountain. The species concerned are *Garrya elliptica*, *Sequoia sempervirens*, *Umbellularia californica*, and species of *Arctostaphylos*. All of these are woody plants with flower buds formed and ready to swell and then open. They have been visible for three or four months. The first species is represented at the top by a single clump which is situated on the southeast sector. This plant, also, is staminate, so that the anthesis, occurring suddenly, is soon past. The second species, the redwood, behaves, so far as its staminate flowers are concerned, in the same way. It does not occur along the "Twenty Minute Walk," but some little distance below.

It is also difficult to follow the condition of the ovulate inflorescence as to exact time of anthesis from mere observation. The California laurel (*Umbellularia californica*) shows typical wave anthesis, scattering at first, then more and more full, arrested if a few cooler days are intermingled with the

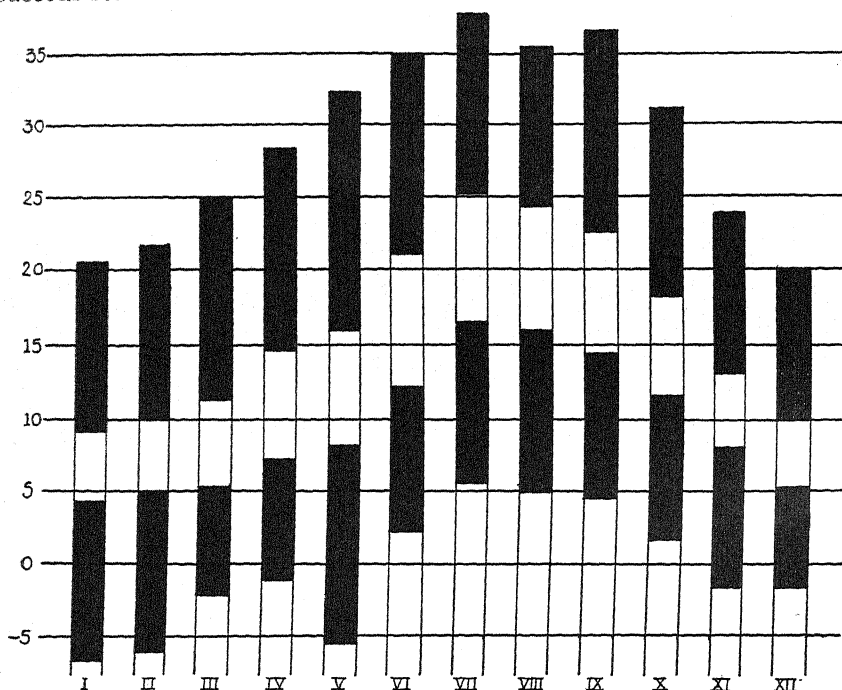


TEXT FIG. 3. The monthly temperature maxima, mean maxima, minima, and mean minima for the period between July, 1922 (VII), and May, 1923 (V), June data for 1923 not being available. The upper solid portion of each column indicates the difference between the mean maximum and the maximum, the central blank portion the difference between the mean minimum and the mean maximum, while the lower solid portion represents the difference between the minimum and the mean minimum. The Arabic numerals represent degrees of the Centigrade scale, while the Roman numerals represent the months as in text figures 1 and 2.

warmer ones, blossoming more freely on the south side of a tree and less so, even decidedly belated, on the north side. It begins its anthesis towards the base of the south slope fully three weeks or a month before it starts at the top. The species of *Arctostaphylos* (four or five of them) are already in full anthesis below and have produced a conspicuous flowering effect before the species (mostly, at least, *A. glandulosa* Eastwood) begins at the top, the lag for the top being about four weeks.

During late January and through February the species mentioned above expand and open their flower buds, the pistils are pollinated, and by early March they are either through or in late anthesis on the south exposure at the top. On the north and northwest sides there is a decided lag of several

(about 3 or 4) weeks. During March, when the temperature (especially in 1923) is somewhat higher, an annual (*Erodium cicutarium*) and an herbaceous perennial (*Galium Nuttallii*) come into bloom, and I am in doubt whether to group them with the first or with the second group. The first arises from seed, while the second is found blossoming only from persistent yet herbaceous stems.



TEXT FIG. 4. The average monthly temperature data for the period July, 1898, to June, 1912, expressed in the same way and with the same arrangement as in text figure 3.

The second really noticeable wave of anthesis occurs in April. The plants at the top in the south sector are: *Castilleja foliolosa*, *Ceanothus sorediatus*, *Dendromecon rigidum*, *Diplacus glutinosus*, *Eriodictyon californicum*, *Lupinus albifrons* (?), *Quercus dumosa*, *Vaccinium ovatum*, and *Whipplea modesta*. All of these are plants distributed over the entire exposed southward slope of the mountain, but the second species, with its related species of *Ceanothus* lower down the slopes, is the only one conspicuous in its anthesis. The lighter or slightly darker blue of these *Ceanothus* species ("California lilacs") makes of the mountain a most beautiful spectacle in April and early May, even more conspicuously effective than the earlier waves of anthesis of the manzanitas (*Arctostaphylos* spp.)

*Ceanothus sorediatus* and, even more conspicuously, *Quercus dumosa* are provided with compact, scaly hibernacula. *Vaccinium corymbosum* also

possesses them, but they are smaller than in either of the first two species. The other six species participating in the April wave of anthesis are only suffrutescent, *Whipplea* being nearly herbaceous, with no scaly hibernacula. All are perennials, and their anthesis is advanced on the lower slopes 3 to 4 weeks ahead of that at the top. *Lupinus (albifrons?)* seems to have come from seed of the season before, and the blossoming begins in its second year.

Preceding the April wave is the small group blossoming in the latter part of March, viz., *Erodium cicutarium* and *Galium Nuttallii*, already discussed as probably belonging with it, and succeeding it in May (late) is a small group, viz., *Chlorogalum pomeridianum*, *Pickeringia montana*, and *Rhus diversiloba*, which are probably the forerunners of the next group, the considerable but not very conspicuous wave of anthesis of June.

The number of species coming to full anthesis in June is larger than that of any month, but none is quite so conspicuous as any one of those participating in the waves of February (*Arctostaphylos* spp.) or of April (*Ceanothus* sp.), since with one exception (*Adenostoma fasciculatum* or "chemise") the flowers make little show and the plants do not cover large areas to the greater or less exclusion of other species.

The list for June is as follows; all but the third are strictly on the southern exposure, the third being more southeastern: *Adenostoma fasciculatum*, *Helianthemum scoparium*, *Holodiscus discolor* var. *ariaefolius*, *Hypericum concinnum*, *Lotus glaber*, *Lotus humistratus*, *Phacelia californica*, *Rhamnus californica*, *Rumex Acetosella*, *Sphacele calycina*, and *Stachys bullata*.

Unfortunately the observations at the weather station on Mt. Tamalpais were discontinued at the end of May, 1923, but for May the mean maximum and mean minimum were 66° and 50° F., respectively (18.8° and 10° C.). The 50° to 66° F. (10° to 18.8° C.) may be responsible here for the group of three species (*Chlorogalum*, *Pickeringia*, and *Rhus*) which blossomed late in May. June, as represented on the average table (from 13 years' observations), has its mean maxima and mean minima between 15° and 20° C., extending above and below a degree or two. The wave of anthesis from late May until late in June may be considered to have its optimum between the mean minimum of 15° C. and the mean maximum of 20° C.

The July wave of anthesis is slight, but from my observations one species, at and very near the Twenty Minute walk on the south slope, viz., *Heteromeles arbutifolia*, the well-known "red-berry" or "Christmas berry," comes into full anthesis during this month. The mean maxima and mean minima of this month are not available for the year of observation because of the cessation of weather observations, but in July, 1922, ranged both somewhat above and somewhat below 20° to 25° C. as to interval between the mean maxima and the mean minima, and *Heteromeles* had just passed anthesis by the middle of August, 1922. In average years the range is from somewhat below 20° C. to barely above 25° C.

The last wave of anthesis of the year is in late August and through September on the south exposure of the neighborhood of the Twenty Minute Walk. There are three species concerned, all Composites, viz., *Baccharis pilularis*, *Bigelovia arborescens*, and *Erigeron miser*. The progress of this wave of blossoming is from above downward on the south slope of the mountains, corresponding to the temperature conditions already alluded to, since in these months the upper portions of the mountain are much warmer than the lower, even hot in the clear sunny weather compared with more or less cool fog conditions below. During the month of September, 1922, the means of maxima and minima were separated by an interval from somewhat below 20° C. to somewhat over 25° C., while on the average July is the hottest month (as it was in 1922) and August and September are somewhat cooler, with nevertheless a fair proportion of average temperature above 20° C. The absolute maxima for late August and September, however, run well above 25° and may have something to do with the wave of anthesis in these cases.

These three species are inhabitants of dry and warm localities, and the second and third are Californian semi-desert types of the interior. The relation to moisture is essentially the same, or at least very similar, in the case of all the species concerned in this discussion. All are xerophytic or with a tendency in that direction. We may think of them all as semi-desert types living in soil which is shallow, on more or less steep slopes, subject to desert insolation and physically dry. A few of the broader-leaved plants, like *Umbellularia* and *Heteromeles*, seem more like mesophytes than such species as those of *Adenostoma*, *Arctostaphylos*, *Diplacus*, and *Eriodictyon*. *Helianthemum scoparium* is very definitely a plant of the semi-desert. All such plants need water to a certain degree, but the succession of their vegetative and reproductive periods is not dependent upon more than a certain amount; in other words, they blossom at the season they do, not because it is the usual season of precipitation, but because of the favorable temperature variable of that season. The same is probable, but not so readily perceptible, in the case of the late summer wave of anthesis of *Baccharis*, *Bigelovia*, and *Erigeron*. These flower at the time of highest temperatures, but also at the time of least precipitation and consequently at the time of least soil and atmospheric moisture. While the January and February group blossoms, as has been observed, whether there has been the usual precipitation or not, there has been no opportunity of observing in a season of unusual summer rains. It seems probable, however, that the moisture variable is of much less controlling character as to the time of anthesis than is the temperature variable.

The last three months of the year show no waves of anthesis and in fact little anthesis of any sort, so that the account of regular full anthesis phenomena may be closed so far as the locality under consideration is concerned. There remains, however, to call attention to a few cases of what may be



designated as sporadic anthesis, that is, of scattering anthesis, which seems to recur with some regularity in the month of August, usually, in our locality and even elsewhere, most noticeable about the middle of the month. The plants most definitely concerned are *Adenostoma fasciculatum*, *Dendromecon rigidum*, and *Pickeringia montana*. The regular and full anthesis of these species occurs in June, April, and May, respectively, and is probably brought about by combinations of temperature such as occur in those months. It is noted, also, that this sporadic anthesis in the case of every one of these three species is more pronounced much lower down on the mountain than it is on the Twenty Minute Walk, where it is barely noticeable, and that the temperatures in August average decidedly under those at the top.

The successive waves of anthesis occur under conditions of successive intervals of higher mean temperatures and of decreasing amounts of precipitation. The text figures show these conditions graphically in the case of each, for the year of observation and as averages for thirteen years. The variation from year to year is much less considerable in the case of the temperature records than in that of the records of precipitation, and anthesis has been observed to lag in connection with a low temperature record and not noticeably in connection with scanty or irregular precipitation. There can be little if any question as to the importance of at least a minimum amount of precipitation for the persistence of the various species under consideration and of all their associates of the upper levels of Mt. Tamalpais, but the date of anthesis is little, if at all, affected by considerable variation in the exact date or amount of precipitation while a cooler period may delay, or a warmer period may hasten, anthesis very noticeably. As has been stated previously, the lag in a wave of anthesis, caused by the difference in temperature on the same exposure, but of several hundred meters' difference in elevation, may amount to three or four weeks, this lag certainly not being due to difference in precipitation but to differences in temperature ( $1^{\circ}$  lower for every 260 meters' increase during the winter and  $1^{\circ}$  higher for every 44 meters increase in summer, intermediate seasons averaging proportionately). There is a very similar difference in lag of blossoming on the north side of East Peak, but situated along the Twenty Minute Walk, behind that of the same species on the south side. The variable of temperature *versus* those of moisture (at least as dependent upon precipitation) and of number of hours of daylight, seems clearly concerned in waves of anthesis and to be closely related to both intervals of mean maxima and mean minima of  $5^{\circ}$ -C. amplitude, with the critical points about  $5^{\circ}$ ,  $10^{\circ}$ ,  $15^{\circ}$ ,  $20^{\circ}$ , and probably also  $25^{\circ}$  of the Centigrade scale. These points seem sufficiently clearly demonstrated by the waves of anthesis, *viz.*, (I)  $5^{\circ}$ - $10^{\circ}$  C., (II)  $10^{\circ}$ - $15^{\circ}$  C., (III)  $15^{\circ}$ - $20^{\circ}$  C., (IV)  $20^{\circ}$ - $25^{\circ}$  C., and (V) possibly from  $25^{\circ}$ - $30^{\circ}$  C.—or the January-February, April, May, June, July, and August-September waves as indicated. This fact falls directly into line with the phenomenon observed in aquatics, in case of which the moisture variable may be entirely

eliminated from discussion, and in cultures, as well as in the behavior at different altitudes, the possibility of a photoperiodic variable being also done away with.

#### SUMMARY

1. There are at least five successive waves of anthesis in the plant community on the south slope of Mt. Tamalpais at about 2400 feet altitude.

2. These waves occur in months when the mean maxima and the mean minima of temperature for the month are approximately 5° C. apart from one another.

3. These conditions in the case of land plants agree with those found in aquatics.

4. The consideration of precipitation conditions and the lag or advance in anthesis on lower altitudes of the same exposure or on different exposures at the same altitude indicate that the temperature variable is the one controlling anthesis, not the moisture variable or the hours-of-daylight variable.

5. All observations on both aquatics and land plants point towards definite points of critical temperature for anthesis, these being at regular intervals of 5° C. from 5° C. upwards to at least 30° C. A slight variation in intensity below or above one of these critical points, if duration is sufficient, may make for or against anthesis or other vital phenomena.

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## THE AGE-AND-AREA HYPOTHESIS WITH SPECIAL REFERENCE TO THE FLORA OF TROPICAL AMERICA<sup>1</sup>

J. M. GREENMAN

The age-and-area hypothesis has been so frequently stated and so generally reviewed that the main thesis is pretty thoroughly understood. For those, however, whose attention has not been especially directed to it I venture to quote, from Willis' recent book entitled "Age and Area," page 63 (1922), his latest expression of the theory.

The area occupied at any given time, in any given country, by any group of allied species at least ten in number, depends chiefly, so long as conditions remain reasonably constant, upon the ages of the species of that group in that country, but may be enormously modified by the presence of barriers such as seas, rivers, mountains, changes of climate from one region to the next, or other ecological boundaries, and the like, also by the action of man, and by other causes.

Stripped of its limitations, however, the hypothesis may be stated as follows: In general the area occupied by a species depends upon its age, or, conversely, the age of a species is proportional to its geographical area.

If this theory can be substantiated and applied generally, it would greatly simplify many problems in plant geography, in the determination of phylogenetic relationship, and in the probable evolution of plant groups.

It is not my purpose in this symposium to discuss the hypothesis with the view of upholding or rejecting it *in toto*, but rather to state briefly the results of several efforts to apply the theory to problems which have come up in connection with my own studies. Hence, that which I have to say will concern primarily a group of plants in which I have been interested for several years, namely, the genus *Senecio*.

In the course of monographic studies of the North and Central American species of the genus *Senecio* I soon found that it was impossible to treat adequately from a taxonomic point of view, as well as from a geographical and phylogenetic standpoint, the Central American species without considering carefully the species of the West Indies and South America. Consequently my work on this genus was extended to the West Indian region, as a whole, and to South America, particularly with reference to specific limitation, geographical distribution, and probable phylogeny.

In this connection, Willis' hypothesis seemed to present a method of attack which might prove helpful. If his hypothesis be true for the flora of Ceylon and New Zealand, then it ought to be applicable to the flora of other parts of the world, at least to a greater or less degree.

Therefore, in order to test some of the principles applied by Willis, I selected three groups of *Senecio*, referable to three sections of the genus

<sup>1</sup> Read in the symposium on "The Age-and-area Hypothesis" at the meeting of the Systematic Section of the Botanical Society of America at Cincinnati, December 29, 1923.



namely, Convolvuloidei, Streptothamni, and Fruticosi. These sections consist of relatively homogeneous groups of species, so that in each case we shall be dealing with groups of at least ten or more allied species in accordance with one of the provisos of Willis. The accompanying outline map will serve to indicate that portion of tropical America with which for the present we shall be concerned, namely, southern Mexico, Central America, the West Indies, and northwestern South America. The three groups of allied species involved are indicated to the left on the map—there being twenty-five species of the section Streptothamni, and fifteen species in each of the other sections, namely, Convolvuloidei and Fruticosi. These plants are all more or less woody; those of the first two sections are vines, and those of the last section, or *Fruticosi*, are low shrubby plants. Structural characters which would facilitate dispersal are practically the same in all cases.

The sections as such have a very extended longitudinal or north-and-south range. For example, the general distribution of the Convolvuloidei extends from the states of San Luis Potosi and Tamaulipas in east central Mexico to northern Chili and Argentina, and is indicated by the succession of small areas outlined in blue; the Streptothamni extend from the valley of Cordoba in southern Mexico and Cuba to Bolivia, as indicated by the successive areas outlined in yellow; and the third group of allied species, the Fruticosi, extends from Costa Rica and eastern Cuba to Peru. Practically all species form a part of the Andean flora, although they are in no sense alpine plants.

The approximate distribution-area of each species is indicated by more or less elliptical, colored and numbered outlines. For example *Senecio confusus* (*S. Berlandieri*) (26) has a geographical distribution covering a certain area in the states of San Luis Potosi, Tamaulipas, and Vera Cruz. *Senecio Hoffmanii* (33) extends over the area outlined in blue in Costa Rica and Panama; and *Senecio Benthami* (41) covers a relatively wide area in northeastern Chili, southern Bolivia, and central Paraguay. Similarly, *Senecio Brittonianus* (16) occupies a geographical range through Peru and Bolivia; *Senecio Freemanii* (25) is known only from Trinidad; and *Senecio trineurus* (21) from eastern Cuba, and so on throughout the entire series.

All outlines indicated on the map are based on actual specimens in the five great American herbaria, namely, the United States National Herbarium, the Gray Herbarium, and those of the New York Botanical Garden, the Missouri Botanical Garden, and the Field Museum of Chicago, supplemented by a field study in southern Mexico and in Central America from Panama to Guatemala. Such a study I realize is open to criticism, because of our very fragmentary knowledge of the flora of South America and certain parts of Central America, particularly Nicaragua and Honduras. Nevertheless, significant results can be obtained by carefully mapping the distribution of related species in this manner.



23. S.  
24. S.  
25. S.

To a certain extent the larger areas of specific distribution are comparable to those species which Willis designated as "wides," and the smaller areas of specific distribution may be compared with the "endemics" of Willis. Although our knowledge of the South American flora is fragmentary, as I have already stated, yet it may be expected that as further botanical exploration is made, the distribution-areas of these species will be extended; but that extension will very likely be more or less proportionate among the several species and will show merely a greater overlapping than is indicated on the present map.

Now, as to whether or not there is any relation here between those species of larger geographical area and those of smaller geographical area which in any way parallels the so-called "wides" and "endemics" of Willis in connection with his work on the flora of Ceylon and New Zealand may be a question worth consideration.

Those species of the Convolvuloidei represented by larger areas in blue, namely, *Senecio confusus* (26), *S. Hoffmannii* (33), and *S. Benthami* (41), present greater stability in morphological characters than do some of the species of the same allied group occupying more limited areas, as, for instance, *Senecio kermesinus* (27), *S. Bernoullianus* (29), and *S. chinotegensis* (30-31). The last species is quite variable, particularly in the northern portion of its range, namely, in Guatemala and southern Chiapas. The same relation appears also to exist between species of larger areas and some of those of smaller areas in the other two groups, namely, Streptothamni and Fruticosi. For example, *Senecio Brittonianus* (16), *S. theaeifolius* (13), *S. teretifolius* (53), and *S. apiculatus* (44), all occupying comparatively large areas, are relatively stable species. Hence, there is a marked indication that those species of larger area are more stable and less subject to variation than the variable species of more limited areas, and perhaps therefore are older. If those species of limited area are younger than those of larger area, the question at once arises as to their probable origin.

It is hardly conceivable that any one species of these three sections, as known today, ever extended over the entire area or even over the area now occupied by any one of the sectional groups of allied species. Thus, to consider these more or less isolated species, which together present an enormous vertical range, as "relic endemics" seems to me quite unsatisfactory, although curiously enough in two sections the number of species is almost equally divided between North and South America.

Any satisfactory explanation of the probable origin and occurrence of related groups of species, such as we have here under consideration, must be on the basis of several combined forces all acting at the same time, not the least of which is the main trend of migration through long periods of time.

It is a pretty generally accepted principle that during cold periods the migration of plants is towards the equator or equatorward, and that in warm periods the direction of migration is towards the poles. It is also a

pretty generally accepted view that since the glacial period there has been a northward migration of southern floral elements, although there is comparatively little definite information on record of a northward extension of South American vegetation.

In the groups of plants under consideration evidence of a northward migration seems to me very marked. The main path of this migration has been, as one would naturally expect, along the main Andean mountain chain into Central America; but at the same time a second path of migration has been northward from Venezuela and Trinidad, through the Lesser Antilles to the West Indies.

The evidence of a northward migration along the Andes into Central America is more pronounced in the case of the Convolvuloidei than in the other sections. The Central American representatives of this group I followed personally in the field from Panama to Guatemala.

*Senecio Hoffmannii* (33), while it extends over a considerable area, is not particularly common in Panama, but may be said to be relatively common in Costa Rica about San Jose, particularly on the lower and southern slopes of the two great cordilleras on either side of the San Jose plateau. That these two great mountain ranges, one to the south of the San Jose plateau, the other to the north of the San Jose plateau, each consisting of one enormous volcanic mountain mass after another, both running almost east and west, constitute a very formidable barrier to a northward migration of plants, is, I think, unquestionable. Those species of this group occurring to the north of the range of *Senecio Hoffmannii* (33) in all probability have originated from *Senecio Hoffmannii* (33) or from a parent species of similar type.

That these plants are northward extensions of a southern floral element is further evidenced by members of the section Fruticosi. For example, *Senecio ledifolius* (*S. vernicosus* var. *major* Wedd.) (43) is relatively common in Colombia, and it also occurs at Buena Vista on one of the cordilleran ranges in Costa Rica, but is not known north of this station. Thus we have here an actual bridging over as it were of South and Central America. Again, *Senecio vaccinioides* (50) is relatively common in northern Colombia, and *S. firmipes* (42), an apparent offshoot of it, is found on the Muerta de la Vueltas, or "Wall of Death" on the southern Cordilleras of Costa Rica. While the last two species are closely related, they can not well be regarded as conspecific. Again, *Senecio teretifolius* (53) of Peru and Ecuador, *S. abietinus* (48) of Colombia, and *S. trichotomus* (56) of eastern Cuba are three allied species evidently from the same line of descent which represent a northward extension into the West Indies.

These two paths of northward migration are even more strikingly shown by several members of the Streptothamni group. Those species indicated by the yellow lines occur all along the Andes from Bolivia to Colombia. About half a dozen species of this group are found on the Cordilleras of

Costa Rica, but only one, or two at most, is found to the north of this great Costa Rican barrier. On the other hand, several species of the *Streptothamni* are found along a line of northward migration from Colombia and Venezuela through Trinidad, San Domingo, Haiti, and Jamaica to Cuba, as represented by *S. Magnusii* (10), *S. decompositus* (7), *S. sanctamartae* (8), *S. scortifolius* (9), *S. Fremanii* (25), *S. domingensis* (23), *S. haitensis* (22), *S. Hollickii* (24), and *S. trineurus* (21).

In conclusion, I may say that a critical analysis of conspicuous and important elements of the flora of tropical America, as represented by three sectional groups of ten or more allied species of the great genus *Senecio*, viewed in the light of Willis' age-and-area hypothesis, lead one to the following conclusions:

1. Each group of allied species presents an extended longitudinal, or north-and-south, continuous range of geographical distribution.

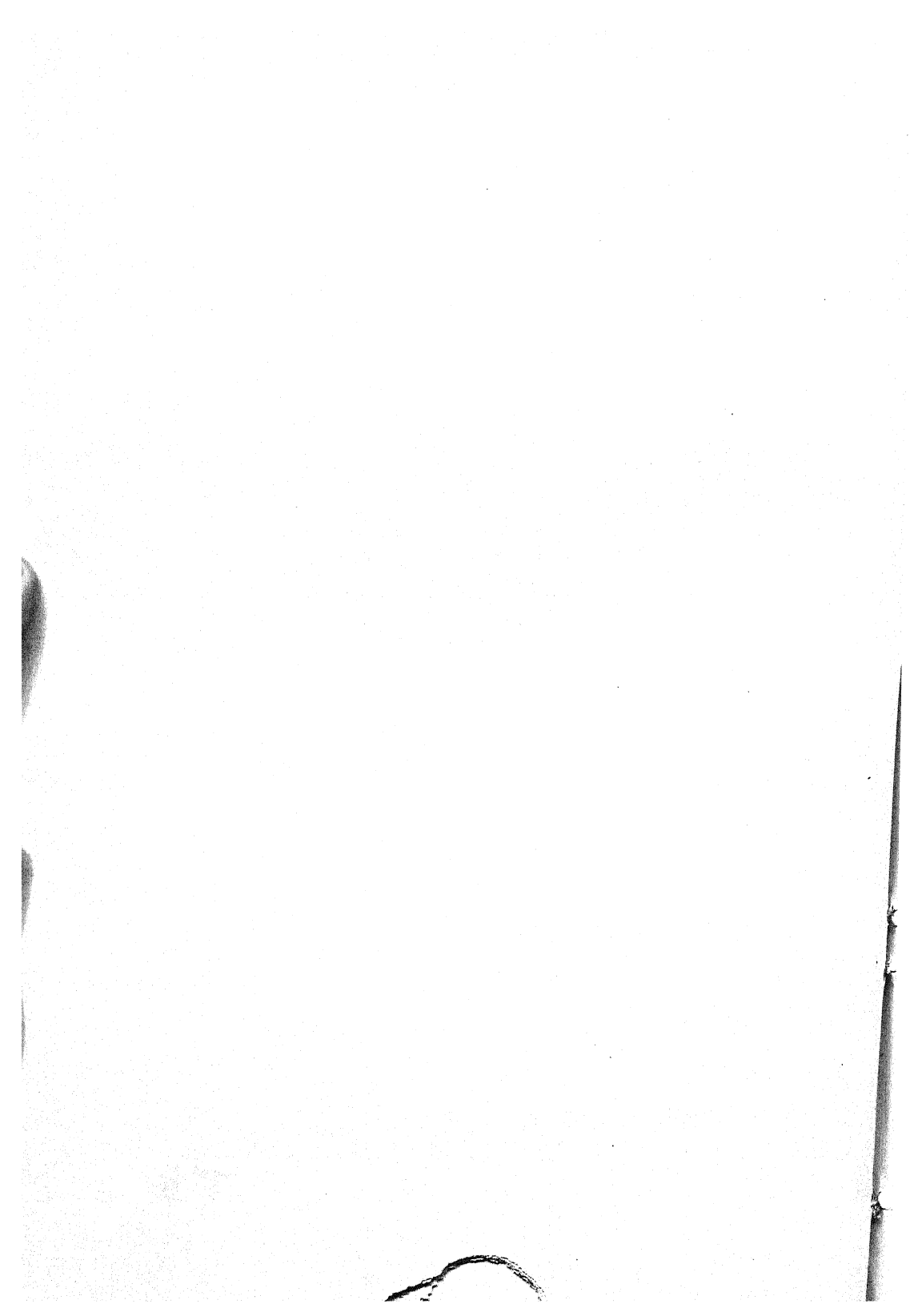
2. There has been a relatively rapid northward migration of species constituting the three sections of *Senecio*, namely, *Convolvuloidei*, *Streptothamni*, and *Fruticosi*; and this migration has extended along two lines of migration as far north as east-central Mexico and Cuba.

3. Species within the same group show a more or less definite relation between the area occupied and the age of the species. This is evidenced by the greater stability in morphological characters of the species occupying large areas as compared with the species occupying small areas—thus according in general with the age-and-area hypothesis.

4. The species of the three groups under consideration are all more or less woody plants, and the majority of them appear to be relatively recent in their origin, at least when compared with other species of the same group or with woody plants of similar groups.

5. The origin of the respective species in the groups of plants specially considered appears *not* to have been through mutation of large size only, as held by Willis for the "endemics" of Ceylon and New Zealand, but rather through natural selection in the course of a relatively rapid northward migration. Mutation of both large and small size may have been a contributing factor, but a factor of comparatively small value.

However Willis' age-and-area hypothesis may be regarded, we must acknowledge that it is fruitfully suggestive and that it has done more to stimulate interest in the study of plant geography than any other contribution in this field since the outstanding contributions of Lyell, Sir Joseph Hooker, and Darwin.



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## TAXONOMIC STUDIES ON THE GENUS *CERCOSPORA* IN THE PHILIPPINE ISLANDS<sup>1</sup>

COLIN G. WELLES

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Numerous plant diseases are caused by fungi which are included as members of the genus *Cercospora*. In the classification of these parasites no attempt is made to separate them on the basis of constant natural characteristics; rather the system of classification is founded on arbitrary factors, in many cases too variable to be of taxonomic value.

No less than fifty-two plant diseases are reported from the Philippine Islands which are alleged to be caused by organisms of this group. The fifty-two organisms occur on about as many hosts. In several cases, however, more than one organism is reported from a single host plant. In many cases organisms which are considered distinct have been reported from hosts which are as closely related as the mung bean and the garden bean. And in the descriptions of these various organisms and of their resultant diseases no notice is taken of fundamental differences. They are separated, frequently, because the reactions of the hosts are noticeably different or because the spores and conidiophores of the fungi vary in their measurements. In other cases, fungi which give nearly the same spore measurements are called identical because host reactions, though on widely separated plants, are similar. Thus, slight differences are used as a means of separation and gross similarities are used as a means of tracing relationship.

The methods just described are used to a great extent, and probably with greater reason, for the fungous forms in the genus *Cercospora*, which have simple conidiophores and tapering conidia. Frequently conidia are encountered which are slightly colored, but in the majority of cases, at least in the forms under discussion, the conidia are entirely hyaline. This slight variation in color has been used to advantage in separating otherwise similar forms.

It was in the hope of throwing some light on the gross features of the

<sup>1</sup> The writer is indebted to Dean Charles Fuller Baker of the University of the Philippines and to Professors E. M. Gilbert and L. R. Jones of the University of Wisconsin for aid given during the preparation of this paper.

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classification of this group of fungi that work was started and conducted in the Philippine Islands during 1921-1922.

#### METHOD OF ATTACK

Work was commenced on the hypothesis that the common *Cercosporas* in the Philippine Islands were, in some cases at least, the same organisms; that host reaction varied in consequence of the function of the host plant in throwing off attack; and that the host range of the parasites was greater than heretofore considered.

For those organisms which show slight if any morphological differences and which cause a variation in host reaction depending on the resistance of the host plant, a method of classification based on more fundamental distinctions than are now used is absolutely necessary.

It may be stated in this connection that lesions caused by the same organism on hosts which have different powers of reacting to the specific stimulus are of very little, if any, value in determining the organism responsible for the malady.

The writer was unable, with but few exceptions, to find conidiophores and conidia on material collected at Los Baños, Laguna, whose measurements closely corresponded to those given by other workers in their descriptions of type material collected locally, or to similar spore measurements which had been recorded in foreign countries. Frequently, also, it was impossible to obtain fruiting structures at a given time which approximated those that had been measured previously in Los Baños on the same host. At the time the measurements were made, it was impossible to give any definite reason for this variation in size. It was conclusive evidence, however, that spore measurements are of no more value in determining true species than is host reaction.

All through the literature, descriptions of *Cercosporas* are given, using spore size and host reaction as a means of differentiating the so-called species. This hypothesis must be limited here, however, to those forms which have slightly colored or hyaline conidia. When there are pronounced morphological differences, such as branching of conidiophores, distinct coloration of conidia, or spores of a decided and constant shape, there is no need of a more fundamental means of separation.

These superficial criteria which are used at present were believed to be poor means of separating species. Later, isolations were made and the organisms were studied in pure culture and in their scope of parasitism. This method of approach had the advantage of showing, by the destruction of the old bases, the more fundamental physiological differences.

#### GROWTH OF ORGANISMS ON CULTURE MEDIA

Organisms isolated from *Phaseolus lunatus* (*Cercospora lussoniensis* Sacc.), *Manihot utilisima* (*C. manihotis* P. Henn.), *Solanum melongena*





(*C. melongenae* Welles), *Allium cepa* (*C. duddiae* Welles), and *Averrhoa carambola* (*C. averrhoi* Welles) were grown on various culture media, including potato-glucose agar, cornmeal, and agar decoctions made from the plants which were parasitized in the field by these particular organisms.

Colonies grown on cornmeal and potato-glucose agar in petri dishes, exhibited approximately the same appearance. At first a rather compact mycelium developed which spread over the surface of the medium quite rapidly at temperatures between 20° and 25° C. After three or four days the growth commenced to develop a grayish-green center. The center kept pace with the growth of the colony, so that there was always a margin of white mycelial weft, about half a centimeter in diameter, surrounding the grayish-green center.

Upon turning the plates bottom-side up, whether the medium was potato-glucose agar or plain cornmeal, the under sides of the colonies appeared very dark green or completely black.

In the grayish-green portions of the colonies the fruiting structures were borne. As conidiophores are merely extended mycelial threads, it was impossible to differentiate them from the mass of mycelium. However, conidia were developed in abundance in from three to five days.

The various fungi developed in a strikingly similar manner on agar slants, host decoctions being used for nutriment. Within twenty-four hours following inoculation, the mycelium formed white patches about one half centimeter in diameter. The growth spread rapidly, and at the end of six to eight days the entire surface of the medium was covered with a thickly matted, grayish-green mycelial weft.

Only one difference was noticed in the manner in which the fungi, isolated from the various hosts mentioned, developed. When a fungus was allowed to develop on a decoction made from the tissues of its respective host plant, there was a slightly more rapid and slightly heavier growth than on any of the other media. Thus, *Cercospora lussoniensis* developed best on a decoction made from the pods and seeds of *Phaseolus lunatus*, *Cercospora duddiae* developed best on a decoction made from onion bulbs, and so on with all the forms mentioned.

From these brief physiological studies there is ample indication that the five species isolated and studied are nearly identical so far as can be judged by their behavior on the culture media used. Spore measurements were not made in all cases, but observation disclosed the fact that the spores were as nearly alike as the eye could judge, both as to size and as to shape and color. Septation was variable within the same limits. It may be mentioned here that all of the organisms were successfully inoculated on the five host plants from which the original cultures were obtained.

#### SEASONAL VARIATION IN SIZE OF FRUITING STRUCTURES

The present method of classifying the various species of the genus *Cercospora* presupposes that neither weather nor host exerts any, or, at

TABLE I. Influence of Seasonal Changes on Spore-development

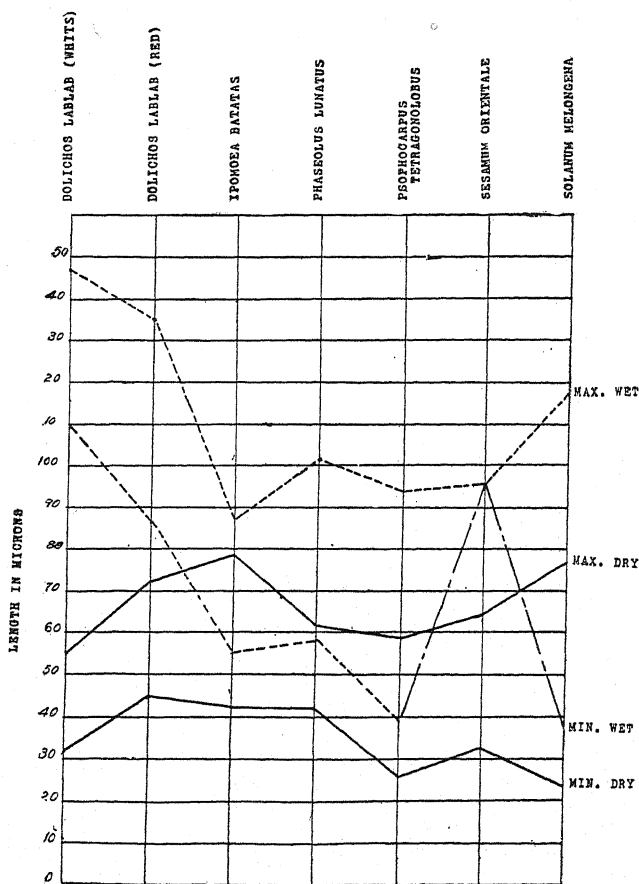
Name of Host and Causal Organism	Date of Measurement	Conidiophores				Conidia			
		Length	Width	Septation	Average	Length	Width	Septation	Average
<i>Dolichos lablab</i> (White variety)	Oct. 10, 1921	79.0-109.0	4.7-7.5	4-6	155.5	110.6-146.9	8.6-15.0	8-14	128.9
<i>Cercospora</i> .....	Jan. 31, 1922	47.4-94.8	4.7-6.3	2-3	65.5	31.6-55.3	3.1-3.90	1-3	39.9
<i>Dolichos lablab</i> (Red variety)	Nov. 6, 1921	*173.8 and 155.7	4.7 and 4.7	6 and 8	164.7	86.9-135.0	11.8-19.0	4-9	106.0
<i>Cercospora</i> .....	Feb. 1, 1922	25.4-125.1	4.3-8.7	3-6	90.6	45.2-71.1	2.8-5.0	3-4	60.3
<i>Ipomoea batatas</i>	Oct. 7, 1921								
<i>C. batatae</i> .....	Feb. 1, 1922								
<i>Phaseolus lunatus</i>	Oct. 10, 1921	90.0-221.2	5.5-6.3	3-6	161.6	55.3-86.9	3.1-6.3	3-6	75.7
<i>C. lussomiensis</i> .....	Feb. 1, 1922	49.4-157.5	5.6-16.4	4-7	103.9	42.0-79.9	4.7-6.7	3-6	63.0
<i>Psophocarpus tetragonolobus</i>	Oct. 16, 1921	63.2-118.5	3.1-6.3	2-4	88.4	59.2-101.7	3.9-11.0	2-9	84.8
<i>Cercospora</i> .....	Feb. 3, 1922	22.1-63.4	4.2-5.3	2-5	46.5	42.9-62.5	4.1-7.1	3-7	51.1
<i>Sesamum indicum</i>	Nov. 20, 1921	*63.0-79.0	4.7-9.4	4-5	71.1	39.5-94.8	3.1-5.5	4-9	75.9
<i>C. sesami</i> .....	Feb. 2, 1922	48.5-153.9	4.5-6.3	3-5	113.2	26.1-59.6	3.6-6.7	3-6	46.5
<i>Solanum melongena</i>	Sept. 20, 1921	30-60	4-6	2-4		†94.8-	7.9-11.1	8-11	94.8
<i>C. melongenae</i> .....	Feb. 1, 1921		Conidiophores absent			32.1-65.7	3.3-4.4	3-5	54.6
						38.6-119	4.6-8.2	3-12	74.8
						23.6-78.1	3.8-7.7	3-8	35.8

\* Small population.

† Measurement lost for extreme.

most, more than a very slight influence on the size and shape of conidiophores and conidia.

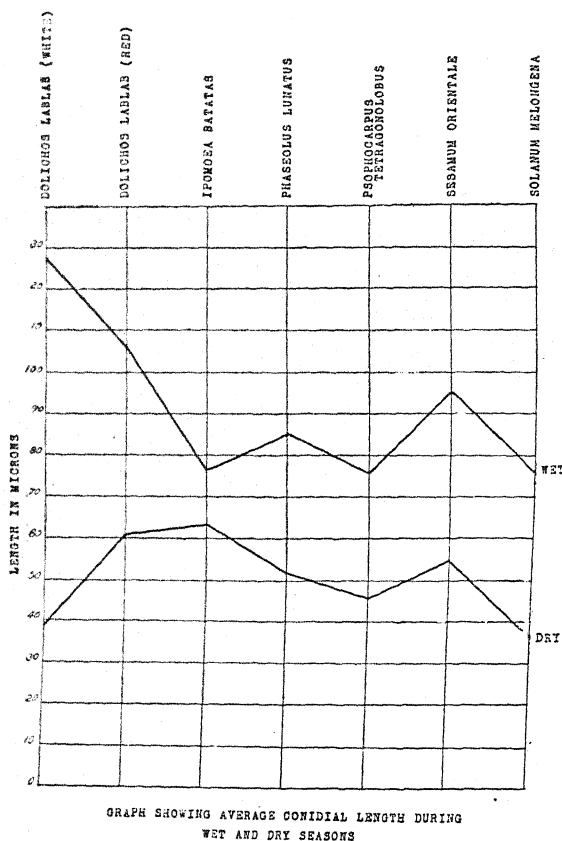
The observations recorded in table I were made at Los Baños in the south central part of the Island of Luzon, P. I. In this locality the wet and dry seasons are very distinct. The rainy season extends, generally, from the first of July to the middle of November, while the dry season begins in January and ends, as a distinct season, in May.



GRAPH SHOWING LENGTH EXTREMES DURING WET AND DRY SEASONS

During the months of September, October, and early November, spore measurements were made from several species of *Cercospora*. Spore measurements were also made of as many of these forms as were available in the latter part of January and early in February. The following plants furnished material during both periods: *Dolichos lablab* (red and white varieties), *Ipomoea batatas*, *Phaseolus lunatus*, *Psophocarpus tetragonolobus*, *Sesamum indicum*, and *Solanum melongena*.

In table 1 the measurements obtained on the various dates recorded are presented. Wherever possible, an average of fifty spores was taken. In the cases of *Dolichos lablab*, red variety, and *Sesamum indicum*, only a few fruiting structures were found, for some unexplained reason, during the rainy season. Unfortunately the average measurement for the dry season for *Sesamum indicum* has been lost and is not again available.



The measurements given in table 1 show a strikingly parallel development for each species. With the exception of the measurements obtained from *Ipomoea batatas*, *Psophocarpus tetragonolobus*, and *Solanum melongena*, the maximum length of the conidia produced during the dry season was no larger, and in many cases was considerably smaller, than the minimum length of conidia produced during the periods of heavy precipitation. The three hosts just mentioned produced strikingly longer conidia in the rainy season than in the dry season.

Conidiophores were not produced by the fungus parasitic on *Ipomoea batatas* either in the rainy or in the dry season. It is an interesting fact

that on *Solanum melongena* rather short conidiophores were formed during the rainy season and that none at all were to be found during the dry season.

In each instance the lesions collected during both seasons were compared, and so far as host reaction is concerned they were identical. Lesions from *Dolichos lablab*, *Phaseolus lunatus*, and *Psophocarpus tetragonolobus* for both periods were taken from the same plantings, and in the case of the parasite on *Solanum melongena* a culture of the organism which was isolated in September was identical with a culture isolated in January.

The measurements of the fruiting structures indicate decidedly that spore size as a means of classification is utterly valueless and misleading when the spores are obtained from uncontrolled field lesions.

#### THE EFFECT OF MOISTURE ON THE SIZE OF FRUITING STRUCTURES

Because in every instance in which a record was made it was found that several species of *Cercospora* formed larger fruiting structures during periods of heavy precipitation than during dry weather, the conclusion was drawn that moisture must play some part in determining the extent and abundance of development. This supposition was strengthened by the fact that in the Philippines fungous diseases are more abundant and luxuriant in their growth during the rainy season.

#### EXPERIMENTAL PROCEDURE

The determination of the effect of moisture on the size of conidiophores and of conidia of *Cercospora* was accomplished during the dry season. The problem at that period was difficult because there were only a very small number of organisms from which to choose, diseases of the leaf-spotting type being dependent on rainfall.

Young leaves bearing lesions caused by *Cercospora*, regardless of host, were selected and used in the experiments. A rather large piece of absorbent cotton, about the size of a fist, saturated with water, was placed in a wax-paper bag which was, in turn, tied over the selected leaf. The leaves were left in this condition for from four to six days, after which they were removed and spore measurements were made.

To admit of comparison, measurements were made of fruiting structures from lesions on leaves of other plants of the respective species, which had been under natural field conditions during the same period. Very little dew fell on these particular days and there was no rain.

Table 2 presents measurements of conidia and conidiophores obtained from spores found on leaves kept at a relatively high humidity and on leaves on which the disease had been undisturbed in its natural development. In each case the moisture supplied by the moistened cotton was exhausted after the third or fourth day, so that the maximum effect of moisture was not obtained.

TABLE 2. *Effect of Moisture on Size of Cercospora Spores*

Name	Date	Natural Infection					
		Conidiophores			Conidia		
		Length	Width	Ave. Length	Length	Width	Ave. Length
<i>C. averrhoi</i> .....	3-22-22	No conidiophores			22.3-54.3	2.4-3.1	42.51
<i>C. batatae</i> .....	3-10-22	No conidiophores			42.4-64.7		55.63
<i>C. nicotiana</i> .....	3- 2-22	No conidiophores			31.6-94.8	2.3-3.9	57.77
<i>C. personata</i> .....	3-29-22	79-154.8	3.1-4.7	III.1	38.7-III.3	2.3-4.7	61.74
Name	Date	Infection under Moist Conditions					
		Conidiophores			Conidia		
		Length	Width	Ave. Length	Length	Width	Ave. Length
<i>C. averrhoi</i> .....	3-22-22	No conidiophores			38.8-75.6	2.4-3.3	59.27
<i>C. batatae</i> .....	3-10-22	43.4-73.2	6.3-6.7	55.7	55.3-82.9	4.7-6.3	72.11
<i>C. nicotiana</i> .....	3- 2-22	57.2-135.2	3.4-4.5	104.59	55.3-142.2	2.1-3.1	87.78
<i>C. personata</i> .....	3-29-22	63-134.2	4.7-6.3	90.46	36.3-184.0	3.1-5.5	76.18

But four species of the desired organisms were available, so absolute conclusions from the results obtained are not advisable. It is quite safe to say, however, from these results, that fruiting structures tend to become longer under conditions of high humidity.

No conidiophores were found under field conditions during the first part of March on lesions caused by *Cercospora averrhoi*, *C. batatae*, and *C. nicotiana*. Upon the addition of water, however, conidiophores were found on lesions caused by *C. batatae* and *C. nicotiana*. The conidiophores found on lesions caused by *C. personata* were slightly longer under dry field conditions than under moist conditions.

In each case the average of fifty conidia was raised considerably, presumably through the effect of the water. The light was cut down to some extent by the bags, but it is reasonable to believe that the change was not sufficient to exert any great influence.

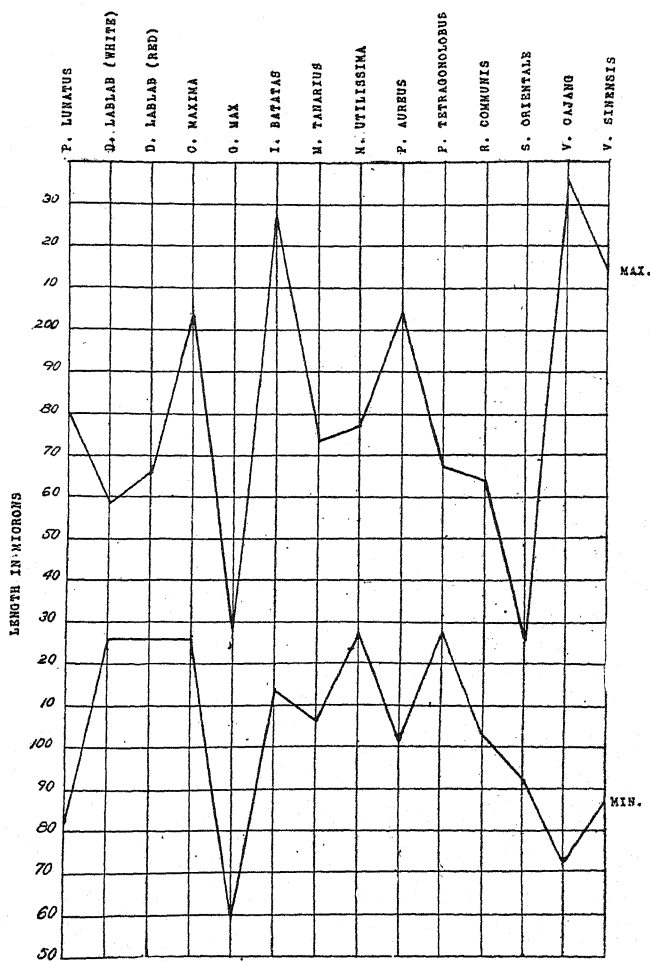
The results given indicate that the larger size of fruiting structures produced during the rainy season is, in a measure, caused by the greater abundance of moisture at that time.

#### INFLUENCE OF HOST ON SIZE OF FRUITING STRUCTURES

Inoculations with pure cultures of *Cercospora lussoniensis* were made on various leguminous and non-leguminous plants. In doing this it was possible to obtain approximately parallel conditions on each host. The plant tissue itself was the greatest varying factor, because moisture was supplied as nearly as possible in uniform amount. The majority of the

inoculations were made during October and the first part of November, so the temperature variations were at a minimum; and it is believed that the average amount of heat received by each plant was not widely variant. However, some slight variation might have resulted from temperature fluctuations.

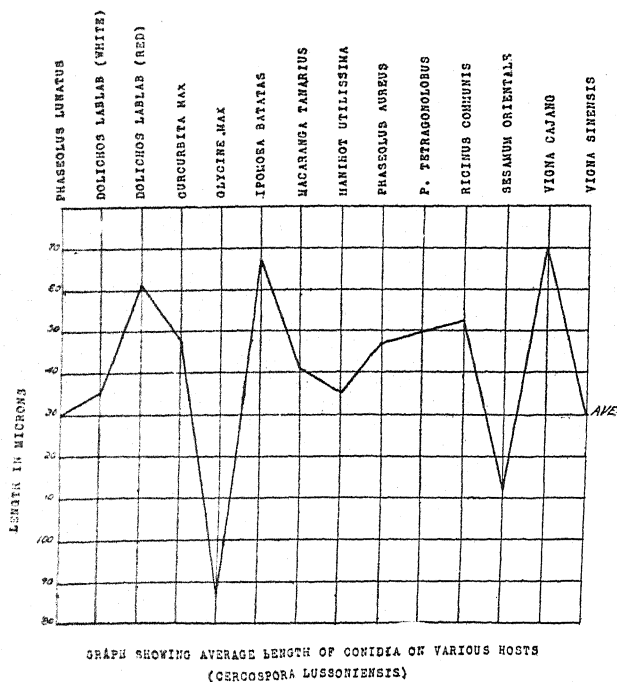
It is rather difficult to account for the great variation of conidial size on the basis of moisture variation alone. The results given here are an



GRAPH SHOWING CONIDIAL LENGTH EXTREMES ON VARIOUS HOSTS

approximation of the effect rather than an accurate quantitative measurement of the absolute influence of the host. Under conditions as they existed in Los Baños at that time more accurately controlled experiments were not possible, since neither moisture- nor temperature-controlling apparatus was available.

The season of the year when the experiments were carried on was probably the most nearly constant in temperature of any period. At any rate, high temperatures for prolonged periods were not experienced. And, because precipitation was at a maximum, moisture was supplied to the fungus in more nearly uniform amounts with greater ease.



GRAPH SHOWING AVERAGE LENGTH OF CONIDIA ON VARIOUS HOSTS  
(*CERCOSPOERA LUSSONIENSIS*)

Table 3 presents measurements obtained from lesions produced on various hosts by artificial inoculations with *Cercospora lussoniensis* which was isolated from *Phaseolus lunatus*. It was not possible to make an absolute determination of this species by cultural studies, but lesions from which the organism was obtained were identical with those in the Baker mycological collection from which the original description was made by Saccardo.

Measurements of conidiophores and conidia made from field infections are included in table 3 as well as conidial measurements from spores produced in pure culture. These, however, are of no value for comparison with the measurements obtained from artificial inoculations. There is little doubt that the water supply in the field, even in periods of greatest precipitation, is considerably less than that furnished by moist cotton in wax-paper bags. The figures presented decidedly bear out this contention. In no case are the spores or conidiophores from artificial inoculations nearly so short as those obtained during the rainy season under natural conditions in the field.



TABLE 3. Influence of Host on Spore-development

Name of Host Plant	Conidiophores				Conidia			
	Length	Width	Septation	Average	Length	Width	Septation	Average
<i>Phaseolus lunatus</i> (Natural infection).....	49.4-157.5	5.9-16.4	4-9	116.6	38.0-62.5	4.1-7.1	3-7	84.8
Pure culture.....					58.7-90.8	6.3-10.6	5-6	75.9
<i>P. lunatus</i> (Artificial inoculation).....	89.5-209.0		2-4	148.6	82.3-180.1		5-9	130.1
<i>Dolichos lablab</i> (White variety).....	227.3-379.2		2-10	302.5	126.4-158.0		8-12	136.8
<i>D. lablab</i> (Red variety).....	127.0-276.5		3-6	221.5	126.5-166.9		5-14	162.8
<i>Cucurbita maxima</i> .....	197.5-395.0		4-10	317.9	126.4-205.4		10-15	147.7
<i>Glycine max.</i> .....	112.9-150.1		4-5	133.5	59.2-128.7		4-10	87.2
<i>Ipomoea batatas</i> .....	213.3-316.0		3-5	266.3	114.5-228.8		5-15	167.5
<i>Macaranga tanarius</i> .....	189.6-323.9		5-7	254.5	107.5-173.8		6-20	141.9
<i>Manihot utilissima</i> .....	110.6-217.2	6.3-7.1	3-6	183.8	118.5-177.9		9-13	135.4
<i>Phaseolus aureus</i> .....	78.2-355.5		4-6	237.5	101.1-205.4		8-13	146.5
<i>Psophocarpus tetragonolobus</i> .....	177.7-316.0		4-6	226.4	118.5-168.2		5-12	136.0
<i>Ricinus communis</i> .....	158.0-316.0		3-6	216.1	103.2-165.9		6-11	142.9
<i>Sesamum indicum</i> .....	150.1-174.8		4-7	163.6	94.8-127.9		6-12	112.0
<i>Vigna cajang</i> .....	165.9-410.8		4-10	326.0	71.1-236.0		5-17	160.8
<i>Vigna sinensis</i> .....	197.5-		5-		87.9-213.3		9-13	130.8

Conidiophores are not so nearly constant in their size as conidia, since they are prolongations of the sub-epidermal mycelium. They are, nevertheless, greatly influenced by differences in the amount of moisture supplied them.

The minimum measurements of conidia on the various hosts ranged from 59.2 microns on *Glycine max* to 126.5 on *Dolichos lablab*, red variety, or a variation of approximately 55 microns. The maximum measurements ranged from 127.9 microns on *Sesamum indicum* to 236.0 microns on *Vigna cajan*, or a variation of approximately 109 microns.

In several instances the conidial measurements were similar enough to justify, in a measure, the present method of classification. However, in the majority of cases, the measurements show no distinct correlation.

From the data presented in table 3 it may be seen readily that the sizes of fruiting structures, induced through artificial inoculation, vary greatly, depending on the host. The results lay no claim to careful quantitative analysis but rather to an indication of the influence exerted by the host plant.

#### COMPARISON OF SPORES PRODUCED ARTIFICIALLY AND NATURALLY ON VARIOUS HOST PLANTS

Inoculations with pure cultures of several species of *Cercospora* were made on more than a dozen host plants which in nature are parasitized by so-called distinct species of the same genus. In the following report a comparison is made between the *Cercosporas* which naturally infect the respective hosts, as reported where measurements and descriptions have been made, and infections induced artificially by inoculation with three isolated species. This comparison shows the ambiguity of the classification used in separating the causal organisms.

No attempt is made here to discuss all forms or all reports of *Cercospora*, but it is the aim to give those forms and reports which have been discussed previously in this paper and which may be found in the more common sources of literature.

In each case, measurements given in the original description of the fungus will be followed by any others which have been found in literature. Next, the measurements made from local material, and lastly the measurements obtained from spores on artificially induced lesions caused by one or more of the isolated organisms, will be given. The fungi used in the inoculation work were obtained from *Phaseolus lunatus*, *Manihot utilissima*, and *Solanum melongena*, and were believed to be identical with organisms described as *Cercospora lussoniensis* by Saccardo, *C. manihotis* by P. Hennings, and *C. melongenae* by Welles, respectively.

### *Cercospora lussoniensis* from Field Infection

The disease of *Phaseolus lunatus* caused by *C. lussoniensis* Sacc. is very common in Los Baños. Many spore measurements have been made from local material. These do not agree with Saccardo's original measurements, and vary according to the season of the year in which the fungus developed.

Saccardo<sup>2</sup> gives the measurements of conidia and conidiophores as follows: Conidiophores 28 to 35 microns in length by 4 microns in diameter; conidia 35 to 45 microns in length by 3.5 microns in diameter, commonly having 3 septa. The measurements of many spores collected in Los Baños during the rainy season fall within the following extremes: Conidiophores 90 to 221 microns long by 5.5 to 6.3 microns in width, with 3 to 6 septa; conidia 59 to 101 microns in length by 4.8 to 8.2 microns in width, with 2 to 9 septa. From spores formed during the dry season the following measurements were obtained: Conidiophores 49.4 to 157.5 microns in length by 5.6 to 6.4 microns in width, with 4 to 7 septa; conidia 42.9 to 62.5 microns in length by 4.1 to 7.1 microns in width, with 3 to 7 septa. (See Pl. XI, figs. 2, 3.)

### Inoculation of *Phaseolus lunatus*

To prove the pathogenicity of the organism isolated, leaves of *Phaseolus lunatus* were inoculated with a pure culture.

The following procedure was used in all the subsequent inoculations. The surfaces of healthy young leaves were washed with mercuric chlorid, one to one thousand concentration, and rinsed thoroughly with sterile water. A mass of mycelium and spores was removed from a pure culture on potato-glucose agar and was placed on the previously treated leaf. Then a wax-paper bag containing a piece of water-saturated cotton, the size of a fist, was tied over the inoculated leaf.

The inoculation was made on September 29. On October 2, upon microscopic examination, mature conidiophores and conidia were found. The following measurements were obtained from these structures: Conidiophores 89 to 209 microns in length, exhibiting 5 to 9 septations (Pl. XI, fig. 1).

It will be observed that the measurements of the conidia are considerably greater than those obtained under field conditions even in the wet season. This is to be expected, because the artificial moisture was supplied during the rainy season and augmented to some extent the natural moisture.

TABLE 4. *Spore Measurements of Cercospora lussoniensis*

	Conidiophores	Conidia	Septa
Field infection. After Saccardo. . . .	28-35 x 4	35-45 x 3.5	3
From Los Baños. Rainy season. . . .	90 to 221 x 5-6.3	59-101 x 4.8-8.2	2-9
From Los Baños. Dry season. . . . .	49-157 x 5.6-6.4	42-62.5 x 4.1-7.1	3-7
Artificial inoculation. . . . .	89-209	82-180	5-9

<sup>2</sup> Saccardo, P. A. Notae mycologicae. Ann. Myc. 12: 314. 1914.

### On *Phaseolus aureus*

No *Cercospora* has been reported from the Philippine Islands on *Phaseolus aureus* with the exception of one, noted by the writer<sup>3</sup> without description. This single one is found abundantly, especially during the wetter portions of the year.

As obtained from local lesions, the fruiting structures measured as follows: Conidiophores 55 to 91 microns in length, with 3 to 5 septa; conidia 51 to 153 microns in length by 6 to 9 microns in width, with 3 to 7 septa.

The measurements recorded by Butler<sup>4</sup> in India are substantially the same as those just presented. According to Saccardo<sup>5</sup> the conidia of *Cercospora cruenta* Sacc. measure 60 to 80 microns in length by 4 microns in width, exhibiting 6 or 7 septa. *Cercospora cruenta* has also been reported on other hosts. A further discussion and presentation of this material will be made under *Vigna cajan*.

The following measurements were obtained from fruiting structures induced by artificial inoculation with *Cercospora lussoniensis*: Conidiophores 105 to 205 microns in length, with 8 to 13 septa; conidia 78.2 to 355 microns in length, exhibiting 4 to 6 septa. Inoculations were made November 13 and the lesions bearing mature fruiting structures were collected November 17.

TABLE 5. Spore Measurements for Organisms on *Phaseolus aureus*

	Conidiophores	Conidia	Septa
<i>Cercospora cruenta</i> . After Saccardo.....		60-80 x 4	6-7
<i>Cercospora cruenta</i> . Los Baños material.....	55-91	51-153 x 6-9	3-7
<i>C. lussoniensis</i> . Artificial inoc.....	105-205	78.2-355	4-6

### On *Dolichos lablab*

Reinking<sup>6</sup> reported a *Cercospora* leaf spot on *Dolichos lablab*. The malady is very common and somewhat destructive on both red and white varieties in Los Baños. The description of the symptoms only was given, so that a comparison of spores is not possible. Because the identity of the fungus has never been demonstrated, measurements recorded by Saccardo will be presented for *Cercospora dolichi* on *Dolichos sinensis* and for *C. cruenta* on an undetermined species of *Dolichos*. The spore measurements obtained from lesions on *Dolichos sinensis* caused by *C. dolichi* are given as follows by Saccardo:<sup>7</sup> Conidiophores 20 to 35 microns in length by

<sup>3</sup> Welles, C. G. A provisional list of the parasitic fungi of the Philippine Islands. Philipp. Agr. Rev. 15: 173, 192. 1922.

<sup>4</sup> Butler, E. J. Fungi and disease in plants, p. 262. Calcutta, 1918.

<sup>5</sup> Saccardo, P. A. Sylloge fungorum 4: 435. 1886.

<sup>6</sup> Reinking, O. A. Philippine economic-plant diseases. Philipp. Jour. Sci. 13: 204. 1918.

<sup>7</sup> Sylloge fungorum 10: 622. 1892.

4.5 microns in width; conidia 50 to 100 microns in length by 3.0 to 5.4 microns in width, with 3 to 5 septa.

The fruiting structures taken from lesions on *Dolichos* sp. caused by *C. cruenta* gave the following measurements according to Saccardo: Conidia 60 to 80 microns in length by 4 microns in width, with 6 or 7 septa.

Measurements made from Los Baños material are as follows: For the white variety, collected during the rainy season, conidiophores 79 to 169 microns in length by 4.7 to 7.5 microns in width, with 4 to 6 septa; conidia 110.6 to 146.9 microns in length by 5.9 to 7.5 microns in width, with 8 to 14 septa. When collected during the dry season the measurements were: Conidiophores 47.4 to 94.8 microns in length by 4.7 to 6.3 microns in width, with 2 or 3 septa; conidia 31.6 to 55.3 microns in length by 3.1 to 3.9 microns in width, with 1 to 3 septa (Pl. XII, figs. 6, 7). For the red variety, during the dry season: Conidiophores 25.4 to 125.1 microns in length by 4.3 to 8.7 microns in width, with 3 to 6 septa; conidia 45.2 to 71.1 microns in length by 2.8 to 5.0 microns in width, with 3 or 4 septa. The following measurements were made from spores of this organism obtained from the red variety of *Dolichos lablab*, no record being made of the period of the year when the collecting was done: Conidiophores 147 to 200 microns in length by 4.7 to 6.4 microns in width, with 6 to 8 septa; conidia 88 to 135 microns in length by 5.9 to 9.2 microns in width, with 4 to 9 septa.

Fruiting structures of the size indicated were obtained from the two varieties of *Dolichos lablab* from artificial inoculations with pure cultures of *Cercospora lussoniensis*. For the white variety: Conidiophores 227 to 330 microns in length having 4 to 10 septa; conidia 128 to 158 microns in length, with 8 to 12 septa (Pl. XIII, figs. 13, 14). For the red variety: Conidiophores 127 to 261 microns in length, with 3 to 6 septa; conidia 126 to 166 microns in length, with 5 to 14 septa.

Both varieties were inoculated October 31 and lesions were collected November 3.

TABLE 6. *Spore Measurements from Organisms on Dolichos lablab*

	Conidiophores	Conidia	Septa
<i>Cercospora dolichi</i> . After Saccardo.....	20-35 x 4-5	50-100 x 3-5.4	3-5
<i>C. cruenta</i> . After Saccardo.....		60-80 x 4	6-7
† <i>Cercospora</i> sp. Los Baños. White var..	79-169 x 4.7-7.5	110-146 x 5.9-7.5	8-14
<i>Cercospora</i> sp. Los Baños. Red variety..	25.4-125 x 4-8.7	45-71 x 2.8-5.0	3-4
* <i>Cercospora</i> sp. Los Baños. White var..	47-94 x 4.7-6.3	31-55 x 3.1-3.9	1-3
<i>C. lussoniensis</i> . Artificial inoc.....	227-339	128-158	8-12

\* Measurements made during the dry season.

† Measurements made during the rainy season.

### On *Psophocarpus tetragonolobus*

The writer was unable to discover any reference to a *Cercospora* disease of *Psophocarpus tetragonolobus* in the available literature. There is one

locally, however, which is common and rather serious in the destruction of its host plants.

The following measurements were made from spores taken from field lesions collected in Los Baños: Conidiophores 63 to 118 microns in length by 3.9 to 6.3 microns in width, with 2 to 6 septa. No record was kept as to the season of the year when these measurements were made (Pl. XII, figs. 9, 10).

When healthy young leaves were inoculated with a pure culture of *Cercospora lussoniensis*, the following measurements were obtained: Conidiophores 177 to 257 microns in length, with 4 to 6 septa; conidia 118 to 188 microns in length, with 5 to 12 septa (Pl. XIV, figs. 20, 21). Inoculations were made October 24 and lesions bearing mature fruiting structures were collected October 27.

### On *Vigna cajan*

*Cercospora cruenta* has been reported on *Vigna cajan* from the United States, and *C. vignae* has been reported on the same host by Ellis and Everhart from Louisiana.

According to Saccardo<sup>8</sup> the conidia of *C. cruenta* found in the United States measure 60 to 80 microns in length by 4 microns in width, with 6 or 7 septa. The same author gives the following measurements for the fruiting structures of *C. vignae* on *Vigna luteola* obtained from Louisiana: Conidiophores 35 to 45 microns in length by 4 to 5 microns in width; conidia 70 to 110 microns in length by 3 microns in width, with 3 to 5 septa.

No attempt has been made to identify the *Cercospora* organism which is commonly parasitic and frequently destructive at Los Baños on *Vigna cajan*. The following measurements have been made from fruiting structures obtained from lesions on this host: Conidiophores 84 to 158 microns in length by 4.3 to 8.6 microns in width, with 4 to 6 septa; conidia 71 to 142 microns in length by 5.5 to 9.4 microns in width, with 5 to 9 septa.

A comparison of the measurements given above shows clearly that the Philippine fungus conforms more closely in the measurements of its spore sizes to *C. vignae* than to *C. cruenta* found in the United States. It would be temerous, however, to make the statement that the local fungus is the same as *C. vignae*. It would be equally temerous to say that the fungus is not *C. cruenta* as described in the United States. It is more probable that the fungus is identical with the latter form, which has been identified in the Philippines on *Phaseolus aureus* by comparison with American and Indian material.

The following measurements were obtained from spores formed on lesions induced artificially with *C. lussoniensis*: Conidiophores 165 to 410 microns in length, with 4 to 11 septa; conidia 71 to 136 microns in length with 5 to 17 septa. Inoculation was made November 5 and lesions bearing mature fruiting structures were collected November 8.

<sup>8</sup> *Sylloge fungorum* 11: 621. 1895.



### On *Vigna sinensis*

Reinking<sup>9</sup> reported a *Cercospora* disease on *Vigna sinensis* from Los Baños, but his report included very little description and no spore measurements. The fungus was figured to scale, and from the drawings of conidiophores and conidia rough measurements were obtained. No material seems to have been kept, and so comparison of any phase of the disease was impossible.

The following measurements were obtained from Reinking's figures: Conidiophores 70 to 80 microns in length, with 8 or 9 septa; conidia 21 to 70 microns in length, with 3 to 5 septa. The season of the year when the measurements were made is unknown.

The measurements obtained for spores from field lesions on *Vigna sinensis* are: Conidiophores 98 to 181 microns in length by 4.1 microns in width, with 6 or 7 septa; conidia 51 to 98 microns in length by 6.7 microns in width, with 5 to 9 septa (Pls. XIII and XIV, figs. 15, 16).

*Cercospora vignae* has been reported from Java on *V. sinensis*, and in all probability this organism is similar to the Philippine fungus reported by Reinking.

Measurements were made from spores obtained from lesions induced artificially by *C. lussoniensis* on *Vigna sinensis*. They are: Conidiophores (only one conidiophore could be found) 197 microns in length, with 5 septa; conidia 87 to 213 microns in length, with 9 to 13 septa (Pl. XIII, figs. 11, 12).

Inoculations were made October 24 and distinct lesions bearing mature fruiting structures were collected October 29.

TABLE 7. *Spore Measurements from Organisms on Vigna sinensis*

	Conidiophores	Conidia	Septa
<i>C. vignae</i> . Java material.....	70-80	21-70	3-5
<i>Cercospora</i> sp. Los Baños. After Reinking.....	98-181 x 4.1	51-98 x 6.7	5-9
<i>Cercospora</i> sp. Los Baños material.....	35-45 x 4-5	70-110 x 3	3-5
<i>C. lussoniensis</i> . Artificial inoculation.....	197.5 x 5	87-213	9-13

### On *Ipomoea batatas*

The leaf-spot disease of *Ipomoea batatas* caused by *Cercospora batatae* was but recently reported by the writer<sup>10</sup> from the Philippine Islands.

Saccardo<sup>11</sup> gives the following measurements for spores of *C. batatae* P. Henn. on *Batatas edulis*: Conidiophores 20 to 30 microns in length by 3 microns in width; conidia 40 to 60 microns in length by 3.5 to 4 microns in width, with 3 to 5 septa.

<sup>9</sup> Reinking, O. A. Philippine economic-plant diseases. Philipp. Jour. Sci. 13: 248. 1918.

<sup>10</sup> Welles, C. G. Two serious plant diseases new to the Philippine Islands. Philipp. Agr. 10: 254. 1921.

<sup>11</sup> Sylloge fungorum 22: 1424. 1913.

Saccardo<sup>12</sup> also reports the following measurements for spores of *C. batatae* Zimm. on *Ipomoea batatas*: Conidiophores 35 to 45 microns in length by 4 to 5 microns in width, with 2 septa; conidia 60 to 100 microns in length by 3 to 4 microns in width, with 4 to 6 septa.

The following measurements were made from Los Baños material: Conidia 55 to 86 microns in length by 3.1 to 6.3 microns in width, with 3 to 7 septa. Although diligent search for conidiophores was made, none were discovered at this time (Pl. XII, fig. 8).

Healthy leaves of *Ipomoea batatas* were inoculated with pure cultures of *C. lussoniensis*. The following measurements were made from the fruiting structures produced: Conidiophores 213 to 316 microns in length, with 3 to 6 septa; conidia 114 to 228 microns in length, with 8 to 16 septa (Pl. XV, figs. 26, 27). Inoculations were made November 14 and lesions bearing mature spores were collected November 17.

It is interesting to note the formation of large conidiophores when they were not commonly produced in nature, and the extraordinary size of the conidia. It will be recalled that, when abundant moisture was supplied by the addition of moist cotton, conidiophores of a larger size were produced by this same fungus. The moisture supplied in the latter experiment was necessarily much less than that supplied after the artificial inoculation. The experiment resulting in the relatively smaller measurements was conducted during bright, clear weather with high temperatures, and the other experiment during cloudy, rainy weather when atmospheric moisture was at the saturation point.

*Ipomoea batatas* was also inoculated with a pure culture of *Cercospora manihotis* isolated from *Manihot utilissima*. Fruiting structures of the following dimensions were obtained from the resultant lesions: Conidiophores 189.6 to 275.6 microns in length by 6.7 to 9.4 microns in width, with 3 to 5 septa; conidia 71.1 to 150.1 microns in length by 8.6 to 11.8 microns in width, with 9 to 15 septa (Pl. XV, figs. 24, 25). Inoculations were made February 3 and lesions were collected on February 7.

The fruiting structures obtained from the inoculations with *C. manihotis* were smaller than those produced by *C. lussoniensis* because the inoculations in the one case were made in November during the rainy season, and in the other instance in February during an exceedingly dry period.

TABLE 8. *Spore Measurements from Organisms on Ipomoea batatas*

	Conidiophores	Conidia	Septa
<i>C. batatae</i> P. Henn. After Saccardo.....	20-30 x 3	40-60 x 3.5-4	3-5
<i>C. batatae</i> Zimm. After Saccardo.....	35-45 x 4-5	60-100 x 3-4	3-5
<i>C. batatae</i> . Los Baños material.....		55-86 x 3.1-6.3	3-7
<i>C. lussoniensis</i> . Artificial inoc.....	213-316	114-228	8-16
<i>C. manihotis</i> . Artificial inoc.....	189-275 x 6.7-9	71-150 x 8-11.8	9-15

<sup>12</sup> Sylloge fungorum 21: 1421. 1912.



### On *Ricinus communis*

The leaf-spot disease of *Ricinus communis* caused by *Cercosporina ricinella* (Sacc. and Berl.) Speg. is very abundant in Los Baños. It may be mentioned that the name *Cercosporina* is applied to some of the Dematiaceae which have colored conidiophores and hyaline conidia. This classification, which was initiated by Spegazzini, has been practically, if not completely, ignored by other mycologists in the naming of new species. All the *Cercospora* forms here discussed would be considered members of the genus *Cercosporina*, if Spegazzini's classification were followed.

The measurements given by Saccardo<sup>13</sup> are as follows: Conidiophores 60 to 70 microns in length by 4 to 5 microns in width, with 1 septum; conidia 90 to 100 microns in length by 4 to 6 microns in width, with 6 or 7 septa.

Measurements as follows were obtained from spores collected in Los Baños: Conidiophores 55 to 110 microns in length by 4.1 to 6.3 microns in width, with 1 to 6 septa; conidia 63 to 169 microns in length by 3.9 to 7.9 microns in width, with 3 to 10 septa (Pl. XIV, fig. 17; Pl. XV, figs. 22, 23).

The following measurements were obtained from spores resulting from artificial inoculation with *C. lussoniensis*: Conidiophores 158 to 316 microns in length, with 3 to 6 septa; conidia 103 to 165 microns in length, with 8 to 11 septa (Pl. XVI, figs. 28, 29). Inoculations were made November 4 and lesions bearing mature fruiting structures were collected November 7.

When *Ricinus communis* was inoculated with a pure culture of *C. manihotis*, structures of the following dimensions were produced: Conidiophores 158.0 to 197.5 microns in length by 6.1 to 7.9 microns in width, with 3 to 6 septa; conidia 72.6 to 98.7 microns in length by 8.6 to 12.6 microns in width, with 8 to 12 septa (Pl. XVI, figs. 30, 31).

TABLE 9. *Spore Measurements from Organisms on Ricinus communis*

	Conidiophores	Conidia	Septa
<i>C. ricinella</i> . After Saccardo.....	60-70 x 4-5	90-100 x 4-6	6-7
<i>C. ricinella</i> . Los Baños material.....	55-110 x 4.1-6	63-169 x 3.9-7.9	3-10
<i>C. lussoniensis</i> . Artificial inoc.....	158-316	103-165	8-11
<i>C. manihotis</i> . Artificial inoc.....	158-197.5	72-98.7 x 8-12.6	8-12

### On *Sesamum indicum* (orientale)

The writer has been unable to find the original or any other description of *Cercospora sesami* Zimm., and therefore is unable to present any measurements of conidiophores or conidia of this species.

Locally, at the time that measurements were being made, the fruiting structures of this parasite were exceedingly scarce. After very careful search only three conidiophores and no conidia were found. The measure-

<sup>13</sup> Sylloge fungorum 22: 1432. 1913.

ments of the conidiophores were: 79, 71, and 63 microns in length by 9.4, 7.9, and 4.7 microns in width, with 5, 4, and 4 septa, respectively.

Conidiophores and conidia obtained from artificial inoculation with a pure culture of *C. lussoniensis* gave the following measurements: Conidiophores 150 to 174 microns in length, with 4 to 7 septa; conidia 94 to 127 microns in length, with 6 to 12 septa. Inoculations were made October 12 and lesions bearing mature fruiting structures were collected October 18.

#### On *Cucurbita maxima*

So far as can be ascertained from literature, no *Cercospora* leaf spot has been reported on *Cucurbita maxima*. Young leaves were readily parasitized by *C. lussoniensis* when the latter was introduced artificially.

The following measurements were obtained from fruiting structures produced on artificially induced lesions: Conidiophores 197 to 395 microns in length, with 4 to 10 septa; conidia 126 to 205 microns in length, with 10 to 15 septa (Pl. XVI, fig. 32; Pl. XVII, fig. 32 A). Inoculations were made October 4 and mature fruiting structures were collected October 8.

#### On *Glycine max*

No *Cercospora* organism is known locally in the Philippines which is parasitic on *Glycine max*, yet *C. lussoniensis* when used as inoculum very readily caused distinct lesions.

The following measurements were made from material obtained through artificial inoculation with a pure culture of *C. lussoniensis*: Conidiophores 112 to 150 microns in length, with 4 or 5 septa; conidia 73 to 128 microns in length, with 4 to 10 septa. Inoculation was made October 8 and mature fruiting structures were collected October 12.

The following measurements were obtained from fruiting structures borne on lesions induced artificially on this same host by *C. manihotis*: Conidiophores 116.1 to 221.2 microns in length by 6.3 to 7.9 microns in width, with 3 to 5 septa; conidia 60.2 to 218.8 microns in length by 7.9 to 10.2 microns in width, with 5 to 16 septa (Pl. XX, fig. 53). Inoculations were made January 26 and mature spores were found January 29.

#### On *Manihot utilissima*

*Cercospora manihotis* P. Henn. is very abundant in Los Baños on *Manihot utilissima* but generally causes very little loss to the crop.

Saccardo<sup>14</sup> presents the following measurements for spores of *Cercospora manihotis*: Conidia 25 to 37 microns in length by 4 to 5 microns in width, with 1 to 5 septa. No measurements are given for conidiophores.

Material collected in Los Baños yielded the following measurements: Conidia 33 to 40 microns in length by 5.1 to 7.9 microns in width, with 2

<sup>14</sup> Sylloge fungorum 22: 1432. 1913.

or 3 septa (Pl. XVII, figs. 33, 34, 37, 38). From the spore measurements the organisms appear to be similar.

No infection of *Manihot utilisima* with *C. lussoniensis* was obtained excepting when the tissue was slightly injured. From such lesions spores of the following dimensions were obtained: Conidiophores 110 to 217 microns in length, with 3 to 6 septa; conidia 118 to 177 microns in length, with 9 to 13 septa (Pl. XVIII, figs. 40, 42). Inoculations were made November 17 and lesions bearing mature spores were collected November 21.

### On *Macaranga tanarius*

According to Sydow,<sup>15</sup> the spores of *Cercospora macarangae* have the following measurements: Conidiophores 100 to 160 microns in length by 3 to 4 microns in width; conidia 55 to 85 microns in length by 4 to 5 microns in width, with 5 to 9 septa.

No measurements were made of spores of *Cercospora macarangae* found locally in Los Baños. The following measurements were made from fruiting structures produced by means of artificial inoculation with *C. lussoniensis*: Conidiophores 189 to 323 microns in length, with 5 to 7 septa; conidia 107 to 168 microns in length, with 6 to 18 septa (Pl. XVIII, figs. 43, 44). Inoculations were made November 25 and lesions bearing mature spores were collected November 29.

### PLANTS NOT PARASITIZED BY CERCOSPORA LUSSONIENSIS

Although many attempts were made to infect *Arachis hypogaea*, *Hibiscus esculentus*, and *Solanum melongena* with *C. lussoniensis*, excepting where the tissue of the leaf was injured these were without success. Infection was quite easily obtained in the case of *Solanum melongena* if, before inoculation, the trichomes were thoroughly rubbed from the surface of the leaf. After this operation, which was actually no more than the removal of a mechanical barrier, the fungus developed as readily as on the other, easily parasitized plants.

### SUCCESSFUL INOCULATIONS ON OTHER HOSTS

The following inoculations, not previously reported, and from which no spore measurements were taken, were successful in causing distinct lesions bearing mature fruiting structures: *Cercospora melongenae* on *Arachis hypogaea* (Pl. XIX, figs. 49, 50), *Dolichos lablab*, white variety (Pl. XIV, figs. 18, 19), *Manihot utilisima* (Pl. XVIII, figs. 39, 40), and *Phaseolus lunatus* (Pl. XI, figs. 4, 5); *Cercospora manihotis* on *Macaranga tanarius* (Pl. XIX, figs. 47, 48), *Manihot utilisima* (Pl. XVII, figs. 35, 36), and *Ricinus communis* (Pl. XVI, figs. 30, 31).

<sup>15</sup> Sydow, P. Diagnosen neuer philippinischer Pilze. Ann. Myc. 12: 575. 1914.

## DISCUSSION

The data presented here show distinctly that the stress placed at present on the sizes of conidiophores and conidia and on the host reaction is misleading in the classification of these parasites. It has been shown that the same organism through its stimulation brings about different reactions on plants which are not very widely separated in their general make-up and relationship.

Whether or not these characteristics are as distinct in all types of *Cercospora*, it is difficult to say. That there is an indication that many of the common *Cercosporas* about Los Baños are identical in their physiological and morphological features is quite certain. It is also true that mycologists have entirely ignored the fundamental distinctive characteristics and have named identical organisms as unrelated parasites.

It is not presumed from this preliminary work that it is best to classify these parasites upon a purely physiological basis. It may be that there are constant morphological differences which are of some taxonomic value. If such exist, they have remained unobserved. It is true, however, that the organisms which are fundamentally different may be separated by their scope of parasitism and by their physiological performances on artificial culture media.


Since the fungus has to make adjustments to the various hosts on which it may be parasitic, little weight should be given to morphological differences such as the size of fruiting structures and even, perhaps, the color of the spores. While there are no data directly available on the latter point, it is well known that light plays an important part in the development of color in the spores of certain rusts. There is no reason to believe that the great differences which exist in the constitution of various host plants will not play some part in the determination of pigment-formation.

It is also shown that there is little reason for the classification of fungi on rather widely separated host plants as similar or identical simply because the host reaction in each case is very similar and the spore measurements coincide.

While it is impossible to suggest any final and elaborate scheme of classification for the genus *Cercospora*, it is possible to state that physiological behavior in relation to host range would give a more accurate and, perhaps, not an altogether clumsy method of arranging these parasites. The fact that these organisms are in most cases easily cultivated artificially, makes it more feasible to use culture and inoculation methods for their classification.

## EXPLANATION OF ILLUSTRATIONS

Credit for the majority of the drawings should be given to C. C. Nacion, a student at the University of the Philippines. The drawings were made with the aid of the camera lucida, at a magnification of about 500 diameters.



## PLATE XI

- FIG. 1. Conidia of *Cercospora lussoniensis* produced artificially on *Phaseolus lunatus*.  
FIG. 2. Conidiophores of *C. lussoniensis* from field infection on *P. lunatus*.  
FIG. 3. Conidia of *C. lussoniensis* from field infection on *P. lunatus*.  
FIG. 4. Conidiophores of *C. melongenae* produced artificially on *P. lunatus*. (See also Plate XII.)  
FIG. 5. Conidia of *C. melongenae* produced artificially on *P. lunatus*. (See also Plate XII.)

## PLATE XII

- FIG. 6. Conidiophores of *Cercospora* sp. from field infection on *Dolichos lablab*, white variety.  
FIG. 7. Conidia of *Cercospora* sp. from field infection on *D. lablab*, white variety.  
FIG. 8. Conidia of *C. batatae* from field infection on *Ipomoea batatas*.  
FIG. 9. Conidiophores of *Cercospora* sp. from field infection on *Psophocarpus tetragonolobus*.  
FIG. 10. Conidia of *Cercospora* sp. from field infection on *P. tetragonolobus*.

## PLATE XIII

- FIG. 11. Conidiophores of *C. lussoniensis* produced artificially on *Vigna sinensis*.  
FIG. 12. Conidia of *C. lussoniensis* produced artificially on *V. sinensis*.  
FIG. 13. Conidiophores of *C. lussoniensis* produced artificially on *Dolichos lablab*, white variety.  
FIG. 14. Conidia of *C. lussoniensis* produced artificially on *D. lablab*, white variety.  
FIG. 15. Conidia from *Cercospora* sp. from field infection on *Vigna sinensis*.

## PLATE XIV

- FIG. 16. Conidiophores of *Cercospora* sp. from field infection on *Vigna sinensis*.  
FIG. 17. Conidia of *Cercosporina ricinella* from field infection on *Ricinus communis*.  
FIG. 18. Conidiophores of *C. melongenae* produced artificially on *Dolichos lablab*, white variety.  
FIG. 19. Conidia of *C. melongenae* produced artificially on *D. lablab*, white variety.  
FIG. 20. Conidiophores of *C. lussoniensis* produced artificially on *P. tetragonolobus*.  
FIG. 21. Conidia of *C. lussoniensis* produced artificially on *P. tetragonolobus*.

## PLATE XV

- FIG. 22. Conidiophores of *Cercosporina ricinella* from field infection on *Ricinus communis*.  
FIG. 23. Conidia of *C. ricinella* from field infection on *R. communis*.  
FIG. 24. Conidiophores of *Cercospora manihotis* produced artificially on *Ipomoea batatas*.  
FIG. 25. Conidiophores of *C. manihotis* produced artificially on *I. batatas*.  
FIG. 26. Conidiophores of *C. lussoniensis* produced artificially on *I. batatas*.  
FIG. 27. Conidia of *C. lussoniensis* produced artificially on *I. batatas*.

## PLATE XVI

- FIG. 28. Conidiophores of *C. lussoniensis* produced artificially on *Ricinus communis*.  
FIG. 29. Conidia of *C. lussoniensis* produced artificially on *R. communis*.  
FIG. 30. Conidiophores of *C. manihotis* produced artificially on *R. communis*.  
FIG. 31. Conidia of *C. manihotis* produced artificially on *R. communis*.  
FIG. 32. Conidia of *C. lussoniensis* produced artificially on *Cucurbita maxima*. (See also Plate XVII.)

## PLATE XVII

- FIG. 32 A. Conidiophores of *C. lussoniensis* produced artificially on *Cucurbita maxima*.  
FIG. 33. Conidiophores of *C. manihotis* from field infection on *Manihot utilissima*.  
FIG. 34. Conidia of *C. manihotis* from field infection on *M. utilissima*.  
FIG. 35. Conidiophores of *C. manihotis* produced artificially on *Manihot utilissima*.  
FIG. 36. Conidia of *C. manihotis* produced artificially on *M. utilissima*.  
FIG. 37. Conidiophores of *C. manihotis* from field infection on *M. utilissima*.  
FIG. 38. Conidia of *C. manihotis* from field infection on *M. utilissima*.

## PLATE XVIII

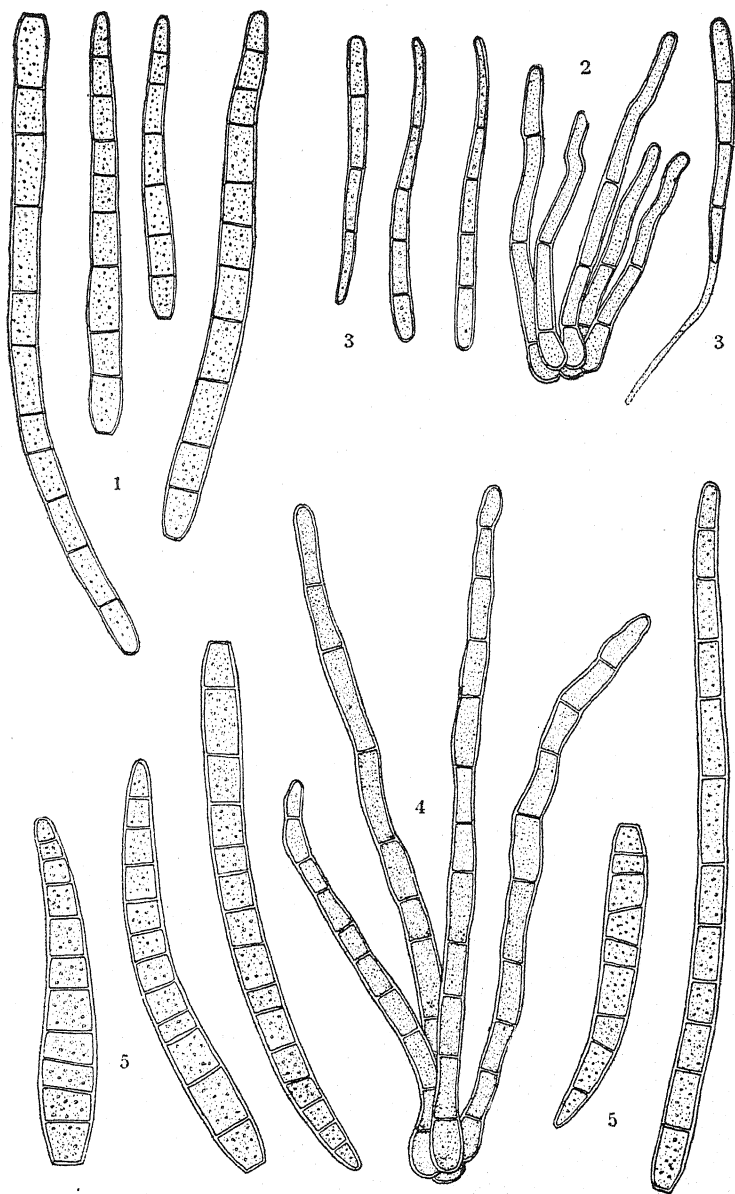
- FIG. 39. Conidiophores of *C. melongenae* produced artificially on *Manihot utilissima*.  
FIG. 40. Conidia of *C. melongenae* produced artificially on *M. utilissima*.  
FIG. 41. Conidiophores of *C. lussoniensis* produced artificially on *M. utilissima*.  
FIG. 42. Conidia of *C. lussoniensis* produced artificially on *M. utilissima*.  
FIG. 43. Conidiophore of *C. lussoniensis* produced artificially on *Macaranga tanarius*.  
FIG. 44. Conidia of *C. lussoniensis* produced artificially on *M. tanarius*.  
FIG. 45. Conidiophores of *C. personata* from field infection on *Arachis hypogaea*.  
FIG. 46. Conidia of *C. personata* from field infection on *A. hypogaea*.

## PLATE XIX

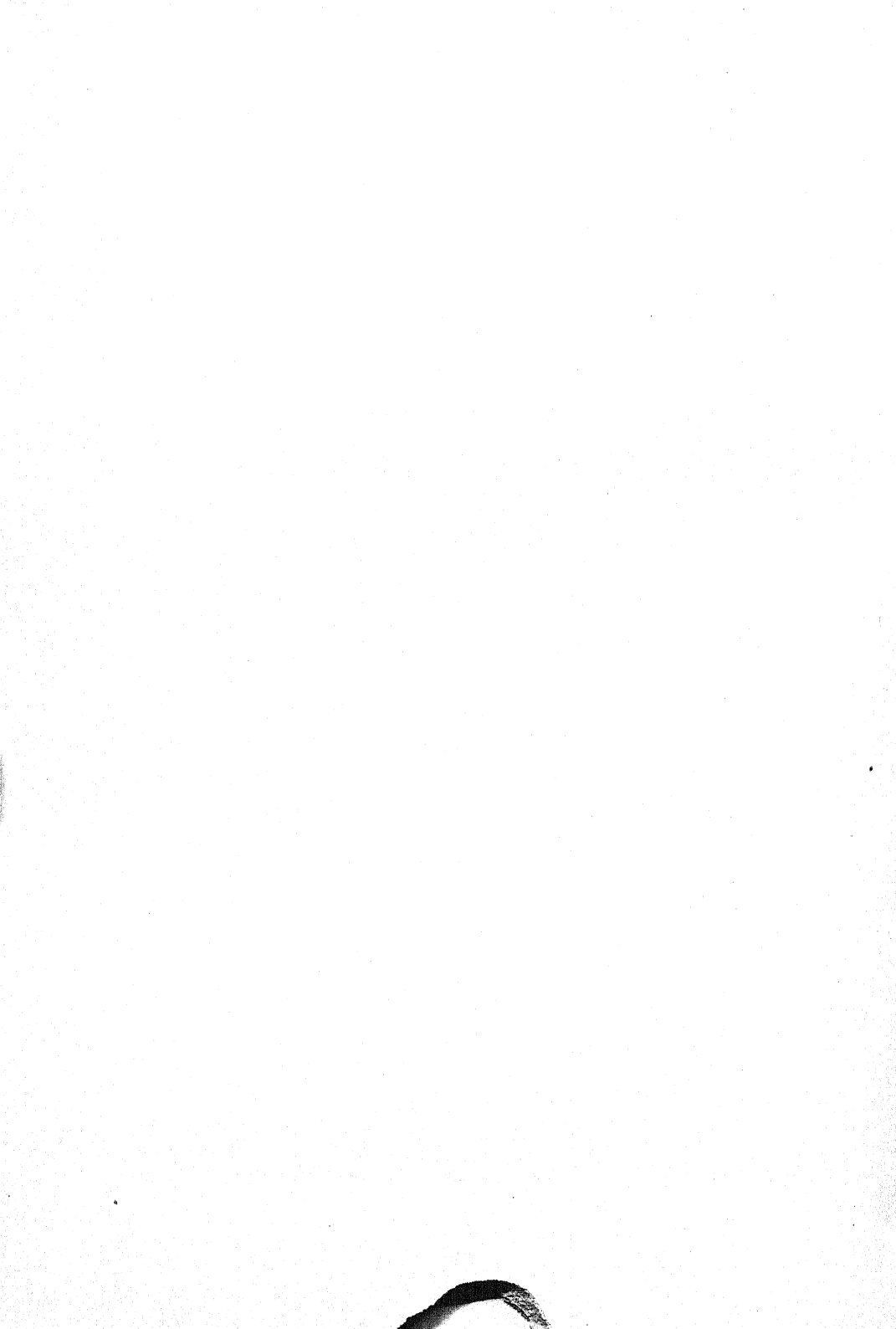
- FIG. 47. Conidiophores of *C. manihotis* produced artificially on *Macaranga tanarius*.  
FIG. 48. Conidia of *C. manihotis* produced artificially on *M. tanarius*.  
FIG. 49. Conidiophores of *C. melongenae* produced artificially on *Arachis hypogaea*.  
FIG. 50. Conidia of *C. melongenae* produced artificially on *A. hypogaea*.  
FIG. 51. Conidiophores of *C. lussoniensis* produced artificially on *A. hypogaea*.  
FIG. 52. Conidia of *C. lussoniensis* produced artificially on *A. hypogaea*.

## PLATE XX

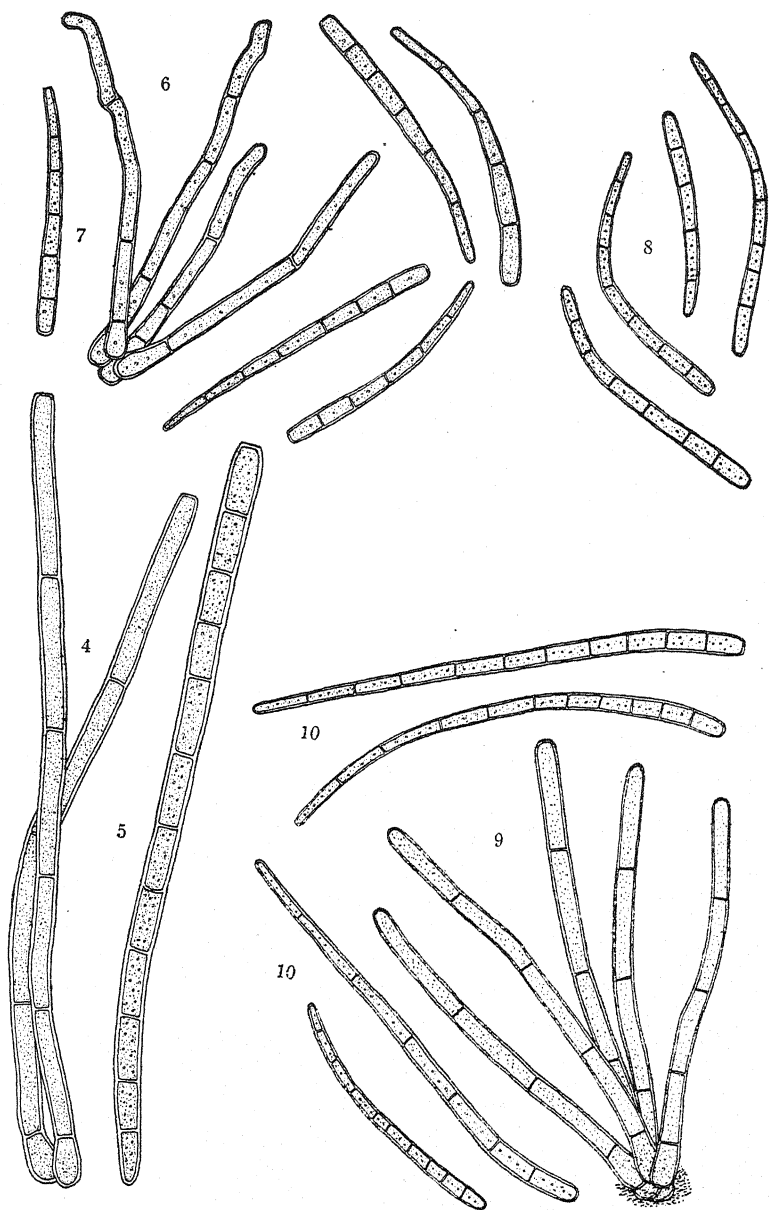
- FIG. 53. Conidiophores of *C. manihotis* produced artificially on *Glycine max*.  
FIG. 54. Conidia of *C. manihotis* produced artificially on *G. max*.  
FIG. 55. Conidia of *C. manihotis* produced in pure culture on potato-glucose agar.



WELLES: CERCOSPORA

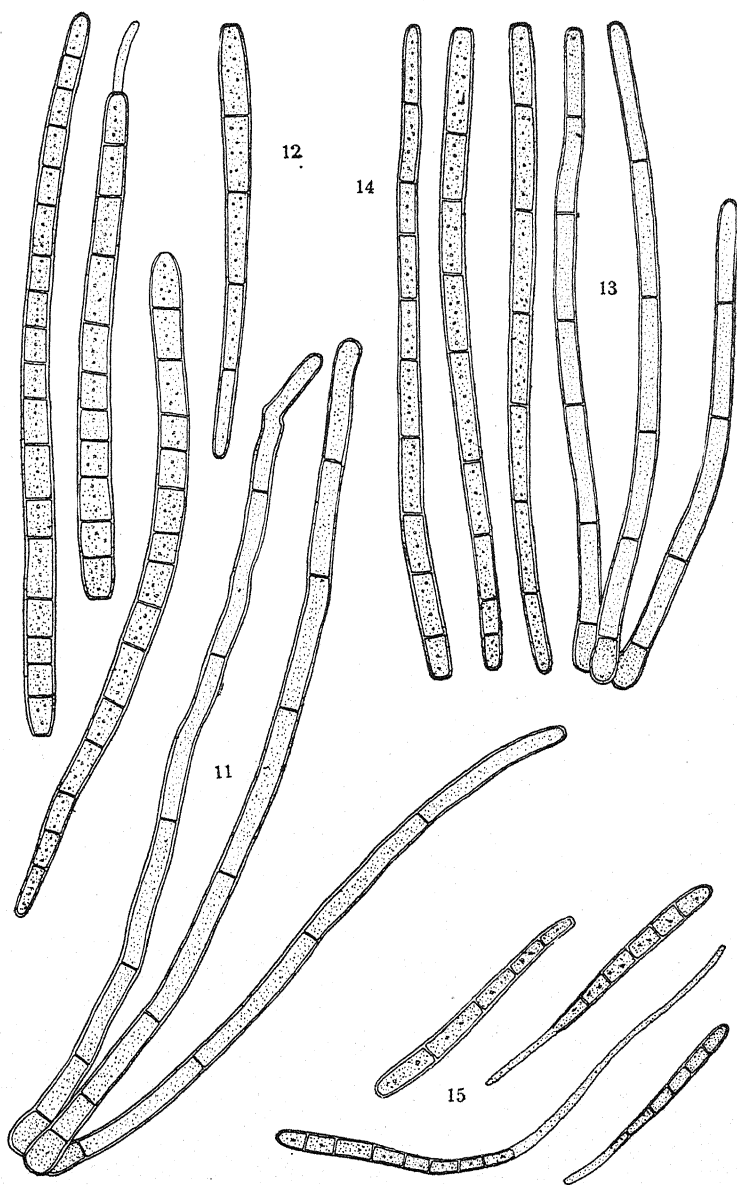






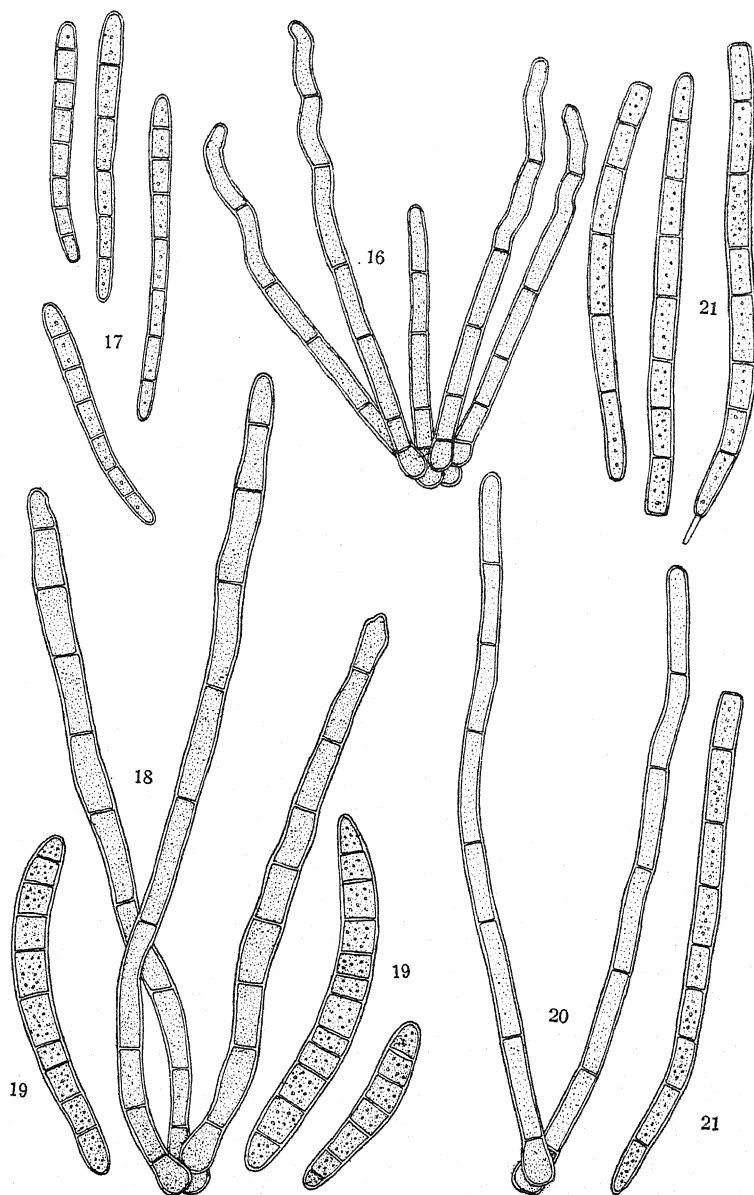
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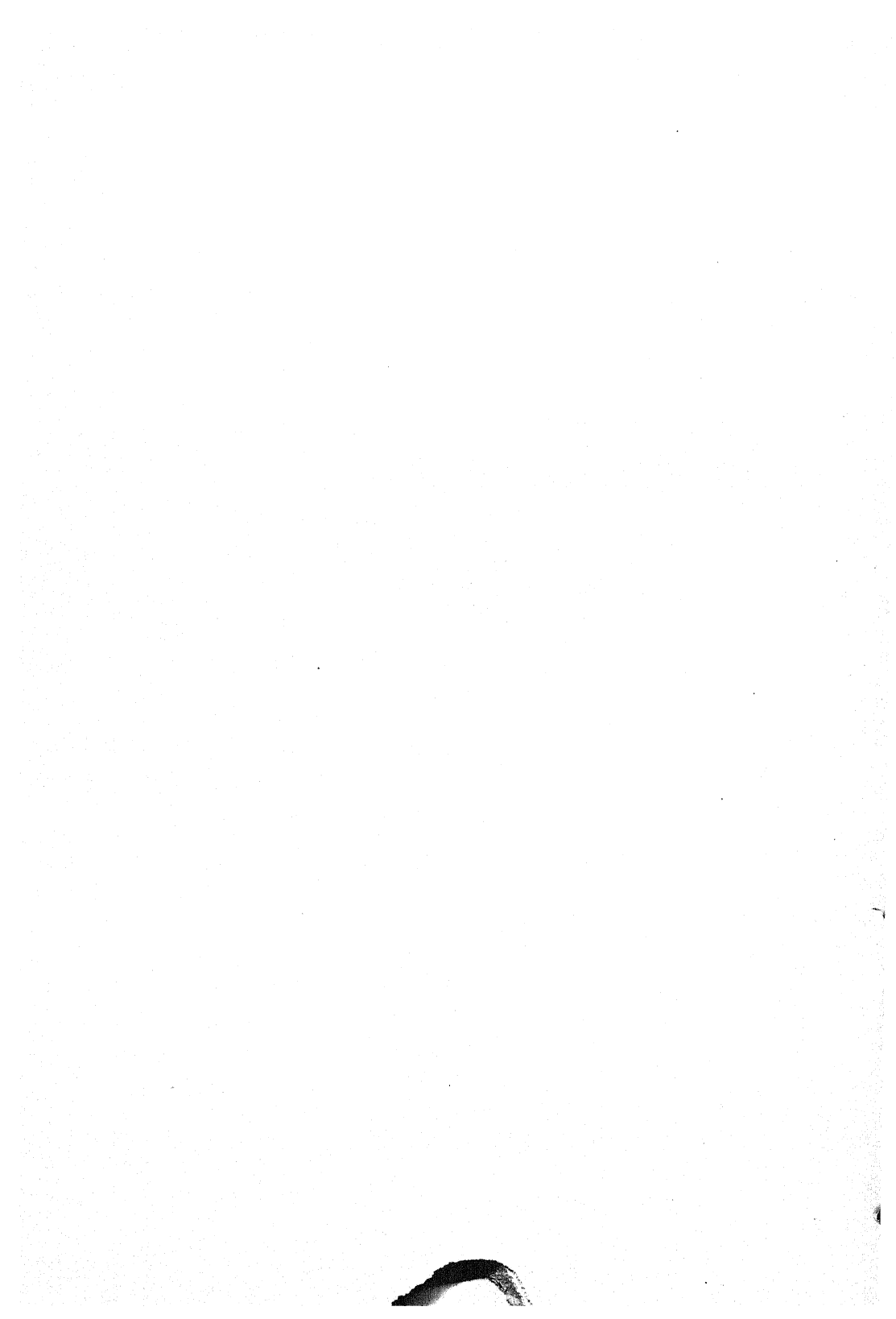


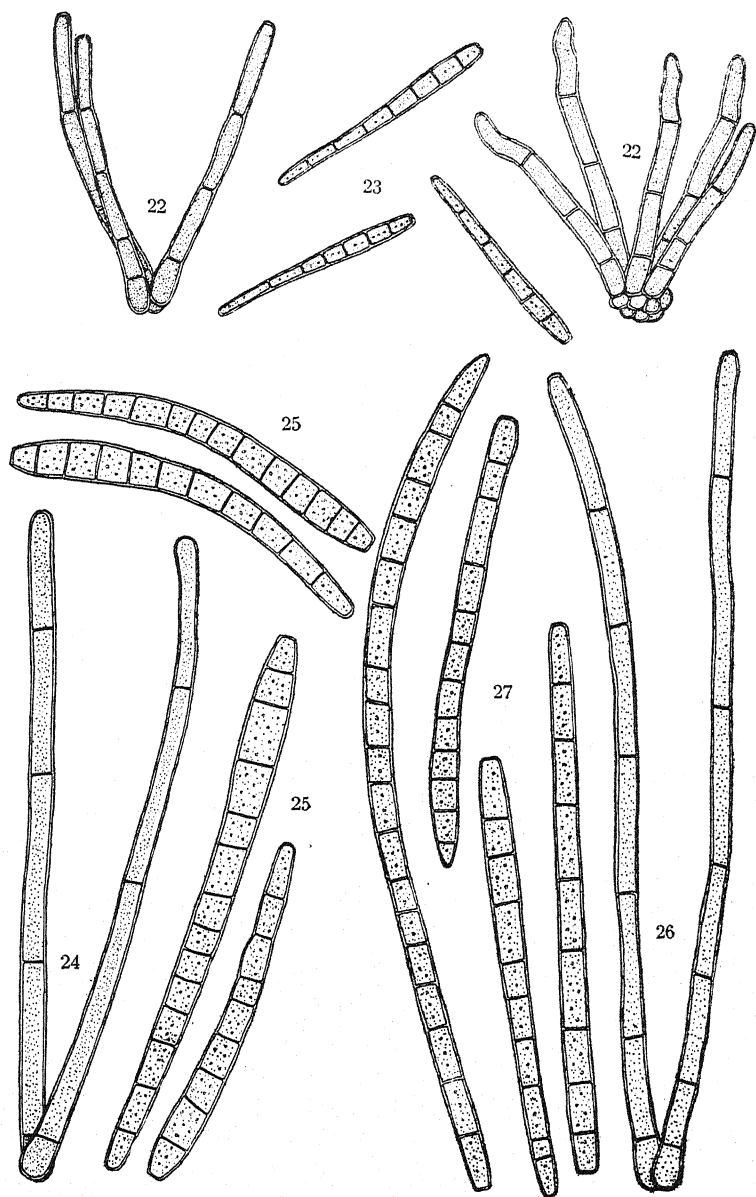
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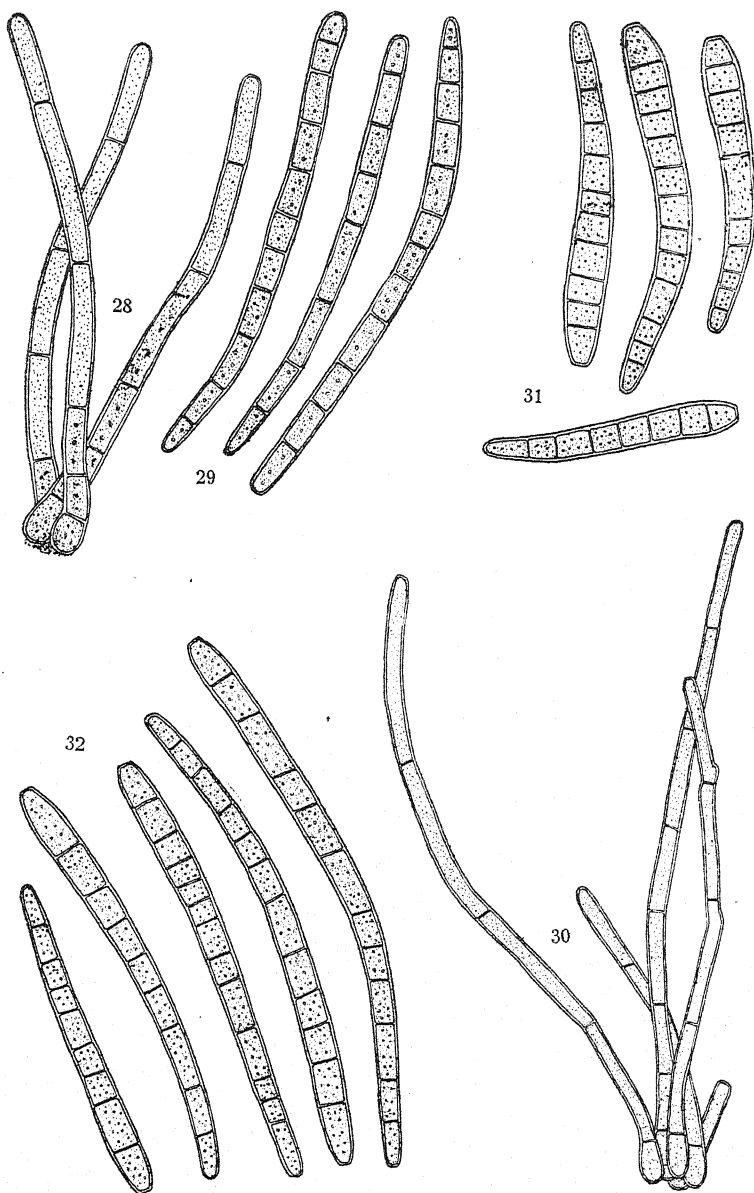




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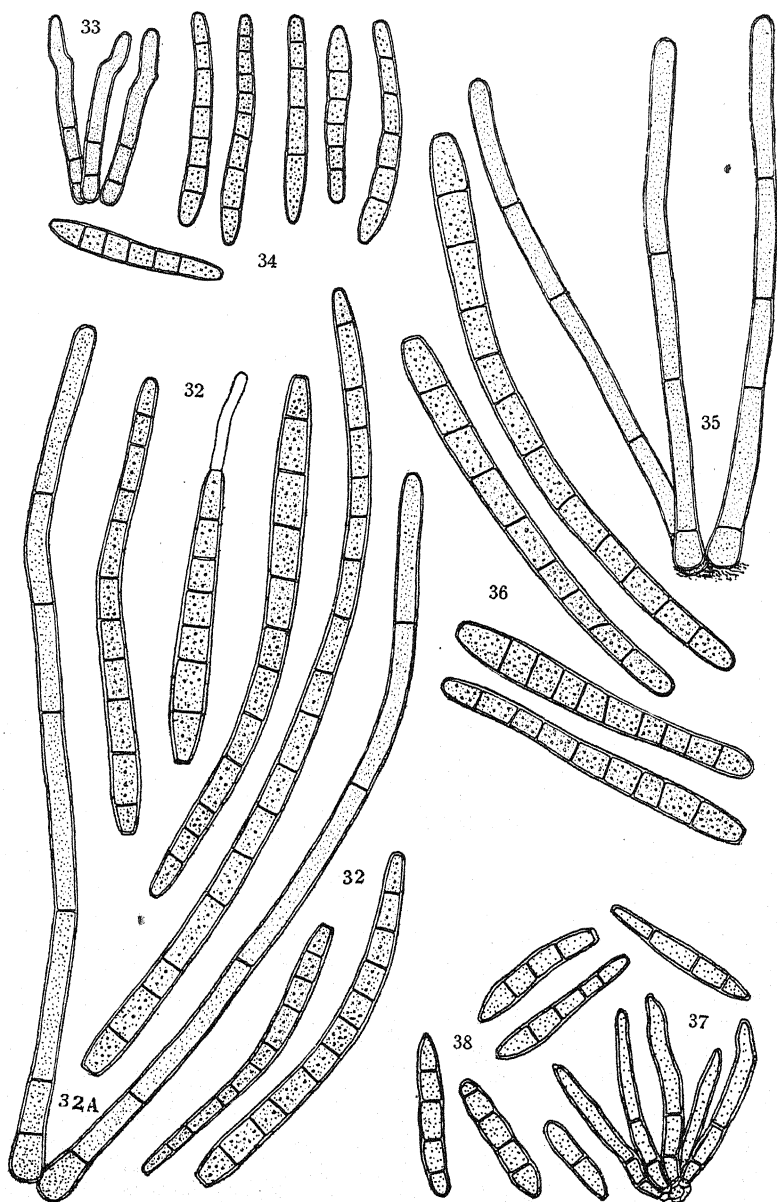






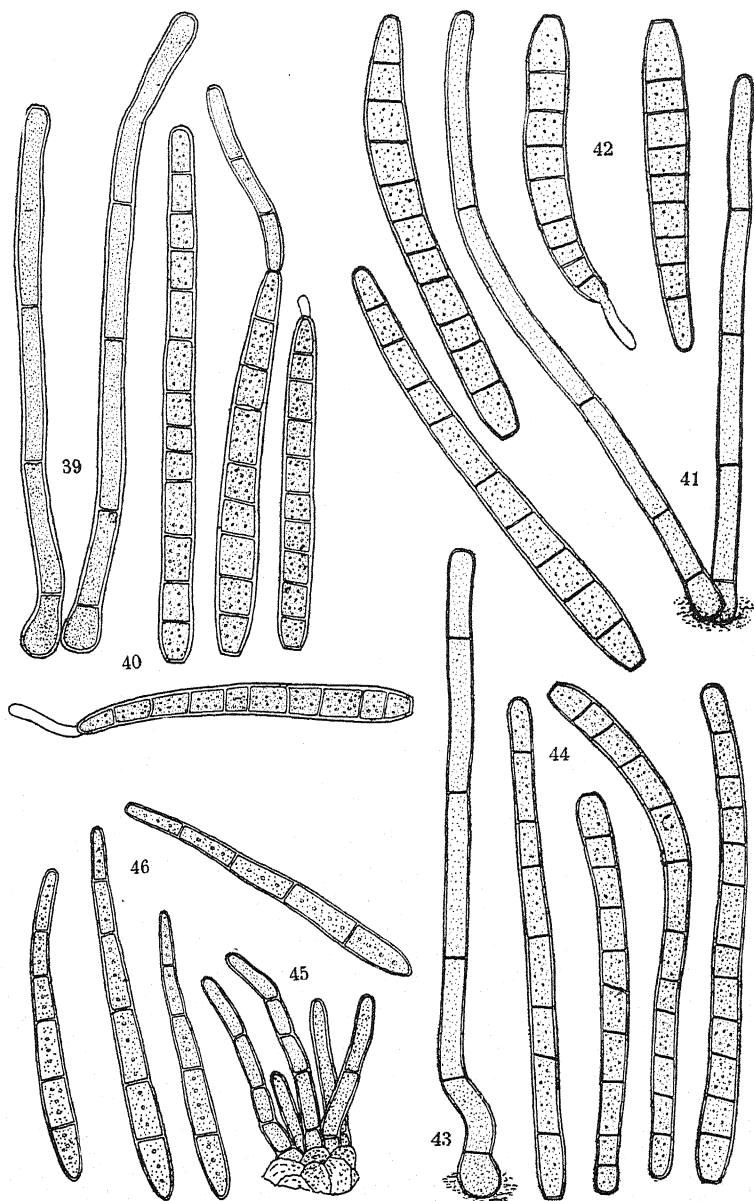
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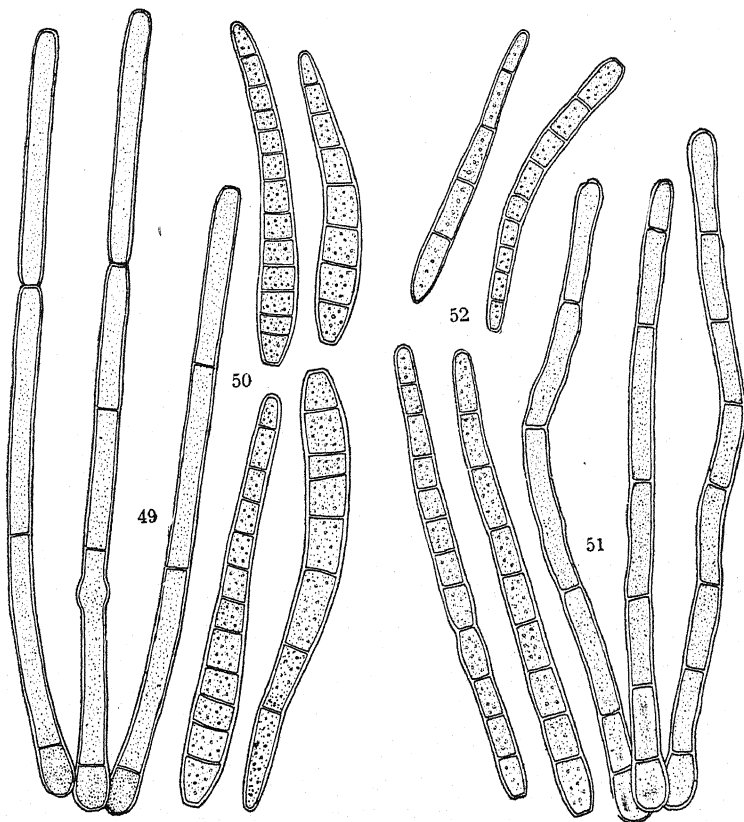
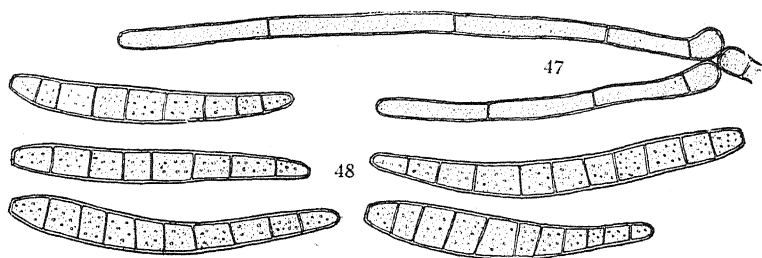
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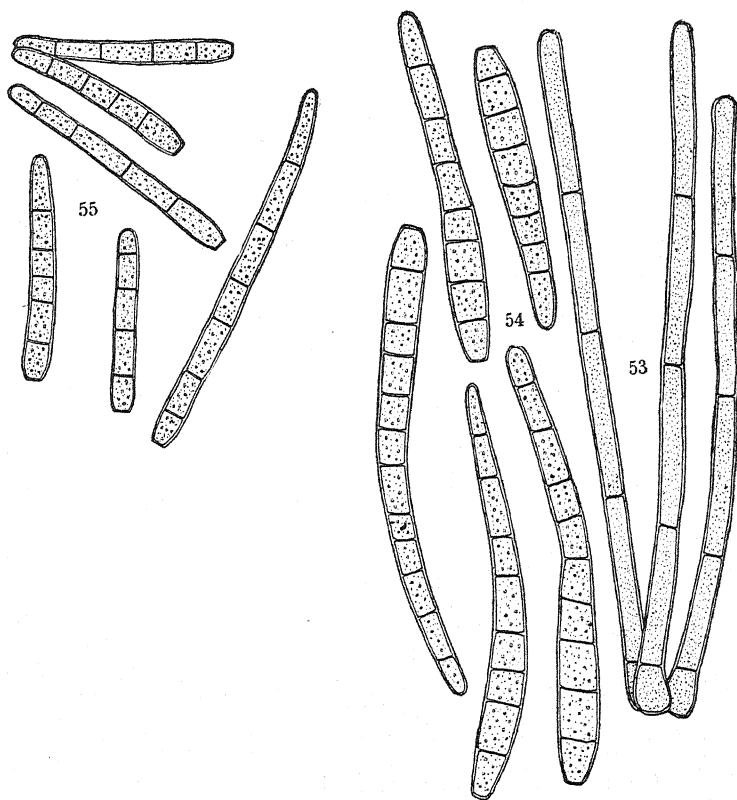




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## CYTOLOGICAL STUDIES ON GASTERIA

### II. A COMPARISON OF THE CHROMOSOMES OF GASTERIA, ALOË, AND HAWORTHIA

WM. RANDOLPH TAYLOR

(Received for publication May 16, 1924)

While working on the form phases of the chromosomes of species of *Gasteria* (3), the question naturally arose as to the possible taxonomic bearing of the points observed. In the microspore-development material, which came from many plants of various species, no distinguishing features were recognized. The root-tip material for sporophytic mitoses was derived from a more limited assortment, and there also no distinctions were noted. Consequently it did not appear that the cytological features would aid in establishing criteria of systematic value in this genus. The natural problem along this line was then the examination of the related genera, of which *Aloë* and *Haworthia* were the most available. These three genera offer very many points of similarity in morphology, and indeed *Aloë* formerly included the other two.

The writer has in previous notes outlined the pertinent literature dealing with chromosome shape (3, 4) as far as it had then become available. Recently a paper by Navašin (1) has come to hand dealing with conditions in *Leontodon autumnalis*. Here he reports two pairs of heterochromosomes (satellites). His figures are not clear regarding fiber-attachment conditions. Newton (2) has prepared a detailed study of chromosome conditions in *Galtonia candicans* and *G. princeps*. For the former he describes fiber-attachment constrictions of the subterminal type and a single pair of satellites, the latter as reported by Navašin in 1912. For the latter species he suggests three pairs of these elements. An interesting feature of this paper is the consideration given to the constrictions in the vegetative prophase, which show conditions comparable to those described by the present writer for *Gasteria* and *Veltheimia* maturation-division prophase. The writer quite agrees with Newton in assigning to the constriction the determining rôle in orienting the attachment of the spindle fiber. However, Newton's incidental statements of the size relations in *Gasteria* and *Aloë* (p. 203) are not borne out by the present studies.

#### OBSERVATIONS

The writer has little to add in the present note over the preceding regarding the chromosome form in *Gasteria* vegetative mitoses. There are seven chromosome pairs, of which three are quite small. The fiber-attachment

constriction in these is closely subterminal. On the shaft of these chromosomes there was described as being present in the second maturation prophase another constriction which was not certainly present at any other phase. In additional material it is fairly obvious in root-tip anaphases (text fig. 2 *I-N*). Of the four pairs of large chromosomes, three have a subterminal fiber-constriction (text fig. 2 *C-H*). One pair has this feature removed from the end by a considerable space, and also bears at the distal

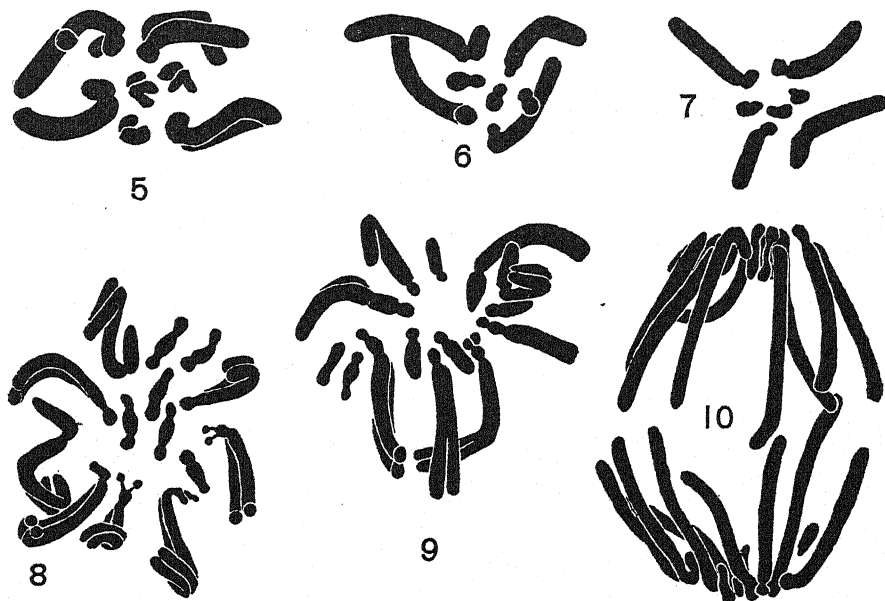


TEXT FIG. 1. Lateral view, anaphase, root-tip mitosis, *Gasteria*. FIG. 2. Selected chromosomes to represent the elements in an anaphase group, *Gasteria*. FIG. 3. Polar view, metaphase, root-tip mitosis in *Aloë arborescens*. The large chromosomes with the shorter head end are marked "s," and a broken chromosome is marked "\*." FIG. 4. Selected chromosomes to represent the elements in an anaphase group, *Aloë arborescens*.  $\times 2000$ .

end a satellite of considerable size (text figs. 1, 2 *A, B*). In this material also the late anaphase chromosomes seem in some cases to be much flattened and with a much paler zone down the middle. These chromosomes are generally twisted, and this pale area follows the twist in such a fashion as to suggest the presence of an anaphase split. Further, the end of the chromosome was often strongly indented and the satellite more or less divided with indications of a double attachment to the main chromosome body (text fig. 2 *A*, etc.), which condition was not seen in earlier anaphase stages.

Two species of *Aloë*, *A. arborescens* Mill. and *A. saponaria* Haw., were studied. The former is a tall species with green leaves margined with whitish spines; the latter is dwarf with spotted leaves and much the ap-

pearance of a *Gasteria* except for the marginal brown spines. So far as was noticed, these have a similar chromosome equipment. The first impression the writer received was that all three genera had the same chromosome form and number. To a limited extent this is quite true, but the representatives of each do exhibit distinct differences. All three have seven pairs of chromosomes, and in all three, three pairs are far smaller than the other four. But in details there are striking dissimilarities. The chromosome complement of the two *Aloës* studied consisted of seven pairs, of which three were quite small with closely subterminal fiber-constriction (text fig. 4 I-N). Of the four large pairs three had the fiber-attachment

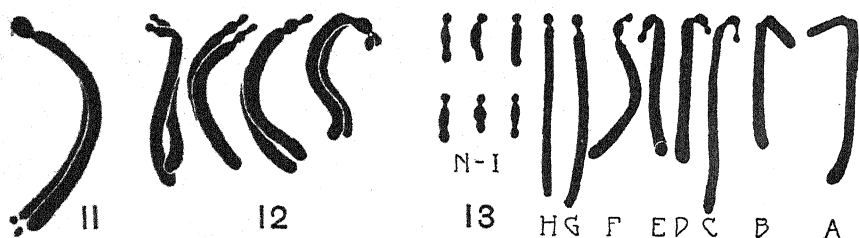


TEXT FIGS. 5-10. *Haworthia cymbiformis*. FIG. 5. Second maturation-division metaphase, microsporocyte. FIG. 6. Second maturation-division anaphase, microsporocyte. FIG. 7. First gametophytic mitosis, anaphase, microspore. FIGS. 8, 9. Polar views, root-tip mitoses. FIG. 10. Lateral view, anaphase, root-tip mitosis.  $\times 2000$ .

quite a little removed from the end, and resembled in this that pair which bore the satellites in *Gasteria* (text fig. 4 C-H). The fourth pair had a subterminal constriction like the non-satellite-bearing type in *Gasteria*, but here by contrast it was the one carrying the satellites on the distal end, which bodies, although they seemed smaller than those in *Gasteria*, differed in no important respect (text figs. 4 A, B; 11).

No maturation material was available for *Aloës*, but a very little was secured for *Haworthia cymbiformis* Haw. (var. *obtusa* Baker?) and root tips were obtained from this species also. The second maturation-division metaphase in the microsporocyte showed seven chromosomes which in

general were very similar to those of *Gasteria* at the same period (text fig. 5). In a few cases the smaller chromosomes appear to be split, but this condition may be an artifact. The anaphase similarly shows one large chromosome with a longer and a shorter arm, three large ones and three small ones with closely subterminal attachments (text fig. 6). The same conditions are present in the first gametophytic mitosis in the microspore (text fig. 7). In root-tip mitoses the three small pairs again correspond to those of *Aloë* and *Gasteria* in all essential respects (text figs. 8, 9, 13 *I-N*). Of the four large pairs a single one has the attachment well removed from the upper end, in which respect this plant resembles *Gasteria* rather than *Aloë*, but this chromosome pair does not seem to be provided with satellites on the distal end as it does in *Gasteria*, in which respect it is more like the three pairs noted for *Aloë* (text figs. 8, 9, 13 *A, B*). The other three large pairs are not all alike, as they were in *Aloë* and in *Gasteria*, but seem to be further differentiated among themselves. One pair has a simple subterminal fiber-attachment of the form found in three large pairs in *Gasteria* (text figs. 8, 9, 13 *H, G*). The remaining two pairs show a tendency toward



TEXT FIG. 11. Metaphase chromosome with satellites, root-tip mitosis of *Aloë saponaria*. FIG. 12. Metaphase chromosomes, root-tip mitosis of *Haworthia cymbiformis*. FIG. 13. Selected chromosomes to represent the elements in an anaphase group, *Haworthia cymbiformis*.  $\times 2000$ .

a double constriction at the upper end. The lower of these—that farther from the end—is the fiber-attachment zone (text figs. 8, 9, 13 *C-F*). The upper constriction shows considerable variety in its prominence, so that the terminal body is sometimes merely a knob on the end, sometimes a ball attached by a short cord. These are variations in appearance often so intimately related to the conditions of fixation or other secondary factors that they should not be stressed. Conditions such as this would link closely the type of non-fiber-bearing constriction found in *Vicia*, *Cyrtanthus*, etc., with that which has been called a "satellite."

#### SUMMARY

In *Gasteria* sps. seven pairs of chromosomes are present: three smaller and three larger with subterminal fiber-attachment, and one large pair with a considerable free arm beyond the fiber-attachment and bearing satellites at the distal end.

In *Aloë arborescens* and *A. saponaria* seven pairs of chromosomes are present: three smaller with subterminal fiber-attachment, three larger with a considerable free arm beyond the fiber-attachment, and one large pair with a subterminal fiber-attachment and bearing satellites at the distal end.

In *Haworthia cymbiformis* (var.?) seven pairs of chromosomes are present: three smaller and one large with subterminal fiber-attachment, one large with a considerable free arm beyond the fiber-attachment, and two large with a double constriction close to the upper end, the fiber attached to the lower end, and the constriction in the short end sometimes being sufficiently pronounced to give the terminal body the appearance of a satellite.

Although it would be unreasonable to assign as generic the characters described or even to assert that other species or even races within the taxonomic range of these individual species might not show some differences of form, it is evident that there is a recognizable form range within the natural group of these three genera, and it is perhaps not unreasonable to expect that a grouping of the species based on chromosome characters would in general correspond to that adopted by the systematist.

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## PRELIMINARY STUDIES ON *PLEOSPHAERULINA BRIOSIANA*

JULIAN H. MILLER

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While studying parasitic fungi causing spots on alfalfa and related plants in the spring of 1922, another very characteristic leaf spot was found, which, under some conditions of temperature and humidity, assumed enormous proportions. At first it was with difficulty that this spot was distinguished from those caused by *Pseudopeziza medicaginis* and *Pyrenopeziza medicaginis*, which were in abundance at that time. However, it was soon seen that there were distinct differences which could be recognized with some study. The *Pseudopeziza* spot is finally irregular in outline, very dark, and always contains one or two raised apothecia. The *Pyrenopeziza* leaf spot is a less sharply defined yellow blotch with numerous pycnidia usually following the venation of the leaf. These spots are more fully described by Jones (5, 6). The new spots tend to be circular with a definite border, always much darker than the light-gray or light-brown interior, 1 to 2 mm. in diameter, and may be few and scattered or so many as practically to cover a leaflet. Under most conditions in the field no fruit bodies are found in them. When the perithecia have been found in the field, they were usually on leaves far advanced in infection and irregularly scattered in larger dead areas containing several of the typical spots. These spots are illustrated on Plate XXI, figures 1 and 2.

Later, upon recognizing the cause of this leaf spot of alfalfa to be an ascomycete, producing large muriform spores in perithecia, it was identified by the writer as *Pleosphaerulina briosiana* Poll. This determination was corroborated by F. R. Jones and J. M. Reade. In searching through the journals very little was found in regard to this fungus in America, and so this investigation was begun in the hope of throwing some light on its development and relationship to its host; particularly to find out just how and under what conditions infection takes place, the extent of its parasitism, and the manner in which this fungus spreads to new fields.

This work has been done in the mycological laboratory of the University of Georgia under the direction of Dr. J. M. Reade, for whose helpful guidance I wish to express my gratitude.

### IDENTITY OF THE FUNGUS

*Pleosphaerulina briosiana* Poll. was first noticed in Europe in 1901 by Pollacci (9) as the cause of an alfalfa leaf spot in Italy. Bubak (1) found the same fungus in Austria in 1909, and Puttemans (10) in Brazil in 1905.



The question was raised by Melchers in Kansas and by Jones in Wisconsin, as to whether the material they had was identical with that described by Pollacci in Italy. In Saccardo's "Sylloge Fungorum" (11) seven species are described, but *P. briosiana* is the only one appearing on alfalfa. Puttemans thought that his material differed sufficiently to deserve being made a separate variety. To settle these points, Jones (3) in conjunction with Melchers (8) sent samples of their infected plants from both states to Europe for comparison with the original, and did establish its identity with *Pleosphaerulina briosiana* Poll.

Samples of alfalfa leaves infected with this fungus were sent by the writer to Dr. Jones, and he pronounced it to be identical with the *Pleosphaerulina briosiana* Poll. in Wisconsin, which he had identified with the original in Europe. So the identity of the material collected here is established.

#### DISTRIBUTION IN AMERICA

The appearance of this fungus was first noted in America by Melchers (8) in 1914 on alfalfa in Kansas, and soon after, Jones (3), working independently, observed it in Wisconsin. Likewise, Jones identified that year specimens from Alabama, Virginia, Iowa, Minnesota, South Dakota, and Indiana. Higgins, botanist at the Georgia Experiment Station, has also recognized it as the cause of a new leaf spot of alfalfa from material sent him from widely separated parts of the state. The writer discovered it here in April, 1922, and since has found it well scattered over the seventy-five acres of alfalfa belonging to the farm of the State College of Agriculture, as well as on many fields in this district. So it is evident that *Pleosphaerulina* has spread over most of this country, wherever alfalfa is raised in any quantity.

#### DESCRIPTION OF THE FUNGUS

The spores are borne in black, carbonaceous, thick-walled perithecia. These arise subepidermally and become partially erumpent at maturity. They are globose to pyriform with short, thick necks and vary from 110 to 140 microns in diameter. In the moist chamber and on culture media mature perithecia producing spores have measured 163 microns. The ostiole is plane and irregular in shape. The perithecia are represented in figures 3, 4, 5, and 6, Plate XXI.

The asci arise from the base of the perithecium, and range from six to eight to the ascocarp. They measure 70-85 x 30-38 microns and are broadly clavate, truncate and smooth at the apex, rounding and narrowing to a pedicellate base. The cytoplasmic content of immature asci is granular and thick, staining deeply with eosin or Congo red, and so far no nuclear details have been made out. The asci open by an irregular tear.

The ascospores are usually borne in biserial order, four diagonally in a row in one direction and four on the other side in an opposite diagonal

direction. Eight spores are always found in the ascus. In the earliest development seen by the writer, they were two-celled by a transverse septum and elliptical to fusiform and hyaline. Later it was observed that all mature spores, that is, those that escape from the ascus in a normal manner, are muriform with three to four septa and one to three longitudinal divisions. They measure 30-40 x 11-14 microns.

#### COLOR OF THE ASCOSPORES

The ascospores of this fungus are a smoky-brown with decided color. All dark-colored spores are hyaline when young, and attain the color only with maturity. These are no exception, as the color is hyaline when found in the perithecium, or even when first expelled. They simply acquire their color late.

Other investigators have thought differently. Pollacci described them as being hyaline, and so it is stated in Engler and Prantl's "Natürliche Pflanzenfamilien" by Lindau (7). Jones and Melchers agree with the original description on that point. To prove this more fully, spores collected on a slide suspended over perithecia were observed when they were first ejected, then every two hours during the day for two days. The slide was in a moist petri dish and exposed to sunlight for at least half of each day.

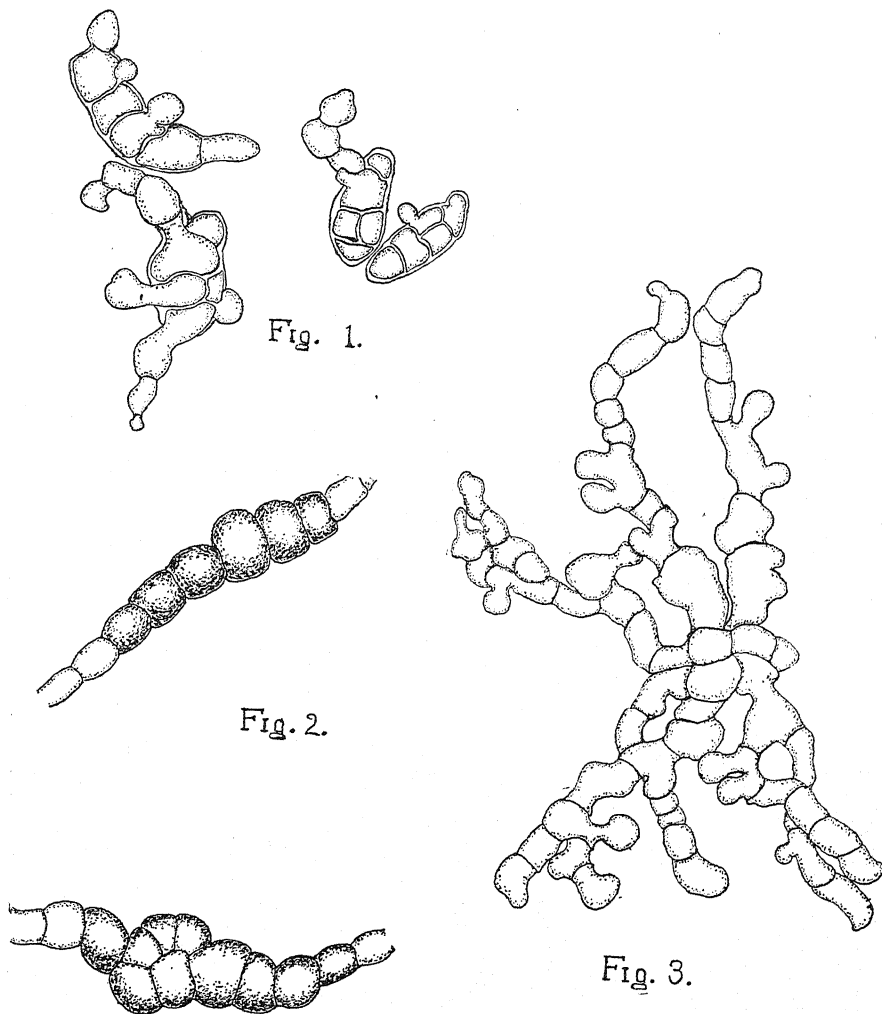
Without exception they were hyaline when first expelled, and gradually darkened until at ten o'clock the second day they were of decided color. Dr. Reade agreed that there was no question but that they were definitely colored. This color was fully as dark as is seen in spores of *Sterigmatocystis niger* Van Tiegh. when viewed singly under the high power.

To determine whether the darkening factor was light or exposure to air, some spores were placed in the dark immediately after expulsion from the ascus. At the expiration of two days these had not developed the color of those left in the sunlight, which fact suggests that light is the factor that causes the production of color.

#### GERMINATION OF THE SPORES

Mature spores will germinate immediately. They do not require a rest period after maturity of the perithecia, as is sometimes the case with other ascomycetes. In all the experiments the spores put out germ tubes equal to the length of the spore in from three to six hours. This was true on agar plates and also in tap water and rain water. They first swell to nearly double size (text fig. 1), then germ tubes appear, sometimes from two cells, or in some cases from five cells, and rarely from all the cells of the spore. The germination of ascospores is shown in text figures 1, 3, 4, and 5.





TEXT FIG. 1. Germination of spores of *Pleosphaerulina briosiana*, three hours after the beginning of swelling. TEXT FIG. 2. Some of the same spores twelve hours later. TEXT FIG. 3. The beginning of a perithecium, indicating apogamous development. No sexual development has been observed.

#### VIABILITY OF THE SPORES

In order to determine how long the spores would remain viable, spores were tested taken from dried material collected on different dates as shown in table 1. This experiment was carried out March 20, 1924.

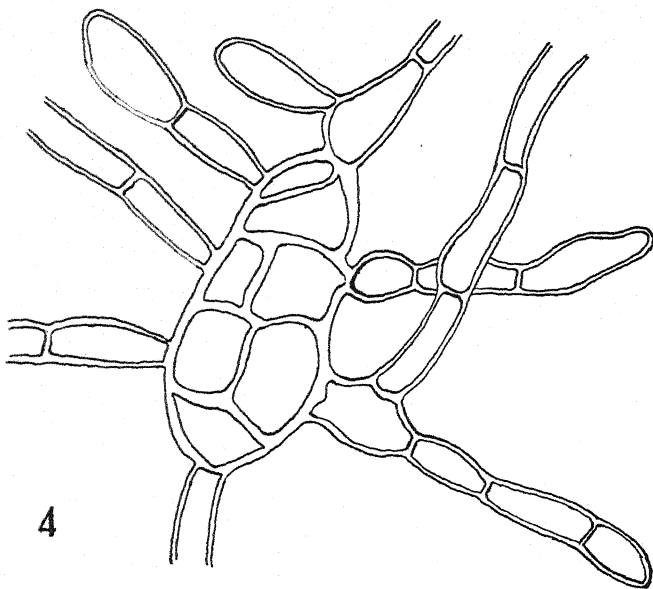
From these results it is seen that some spores will germinate when kept under dry conditions for approximately a year.

TABLE I

Perithecia Collected	No. Spores	Time for Germination	Percentage Germinated
April, 1923.....	10	24 hours	30
Oct., 1923.....	10	24 hours	50
March, 1924.....	10	3 hours, 10 min.	100

## CONDITIONS FOR EJECTION OF ASCOSPORES

Alfalfa leaves covered with perithecia were placed in a moist petri dish with clean cover at nine A.M., and at the end of each hour the top was taken off and replaced by a clean one. This dish was placed in an east window in the morning, and changed to other windows in the building to keep it exposed to sunlight all day. Upon examining the covers it was found that the maximum spore-ejection took place in the morning between nine and ten o'clock. Practically no spores were ejected during the hot part of the day, and a very few towards evening.



TEXT FIG. 4. Perspective view of germinating ascospore of *Pleosphaerulina briosiana*, drawn from a microphotograph.

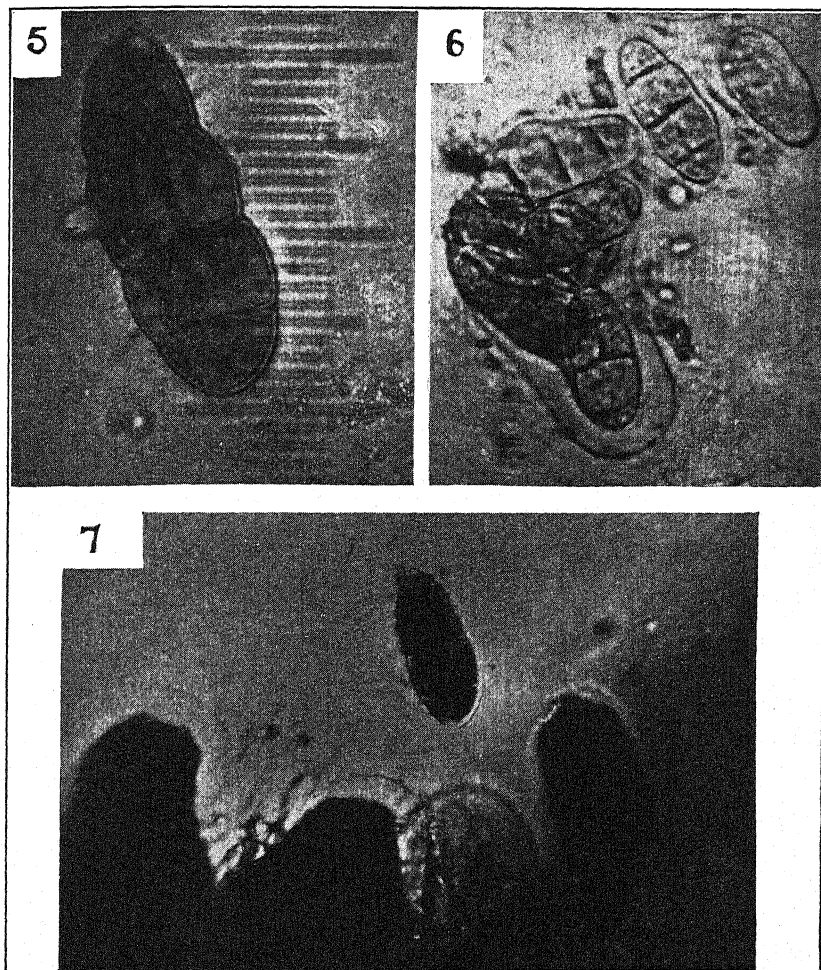
This experiment was repeated with the same results the next day, which was bright in the early morning, and cloudy for the rest of the day.

The results would suggest that most of the spores are expelled from leaves moistened by the night dew and coming in contact with the drying effect of the early morning sunlight.

Further to test the correctness of this theory, perithecia were thoroughly wet in the afternoon, and then placed in a drying oven; an immediate discharge of spores was obtained. Evidently the factors in spore-discharge are the inflation of the ascus, followed by a contraction of the outer wall due to a slight drying, which causes the contents to burst out with force.

#### HEIGHT TO WHICH ASCOSPORES CAN BE EJECTED

Leaves containing mature perithecia were placed in a moist petri dish, as in the experiment above described, and exposed to sunlight at nine A.M.



TEXT FIG. 5. Photomicrograph of germinating ascospore, stained in weak methylene blue, and photographed through Leitz objective 12, ocular 12, with oil immersion. TEXT FIGS. 6 and 7. Photomicrographs of ascospores, just teased out of the ascus and stained with iodine. Leitz objective 6, ocular 12. Note the thickness of the ascus wall.

Different covers were substituted every twenty minutes, and the retired ones were examined for spores. Each time a new one was put on it was placed at a different height above the leaf.

The results are summarized in table 2.

TABLE 2

Height above Perithecia	Diameter of Spore Group
1 centimeter.....	8 millimeters
3 centimeters.....	12 millimeters
6 centimeters.....	50 millimeters
10 centimeters.....	70 millimeters

It follows that from perithecia on leaves on the ground the alfalfa plants may be sprayed with the spores.

#### PHENOMENA INCIDENT TO SPORE-EJECTION

At a time in the early morning when the ascospores were being expelled abundantly, the dish was uncovered and placed under the low power of the microscope and watched closely for about twenty minutes.

The process of spore-ejection could be seen easily. First the tip of the perithecial neck would be ruptured irregularly and the top of the ascus, which had inflated, protruded through this opening. Almost instantly the top of the ascus would burst by an irregular slit, and the ascospores would be shot out.

#### PHOTOTROPIC REACTION OF THE ASCUS

To determine whether or not the light had any influence on the direction in which the ascospores were shot, a culture in a petri dish was placed in an east window at nine A.M. The perithecia were in a spot about three millimeters in diameter. A dot was placed directly over the center of this infected spot on the outside of the dish cover.

About twenty minutes later the top was examined and the center of the spore group was found to be about 3 mm. from the dot directly towards the sun, and practically no spores were found on the opposite side of the dot. Then the dish was reversed and the spore group was again found on the side towards the sun. This experiment was repeated three days in succession with the same results.

From these results it would seem that the direction of the light has a definite influence upon the direction in which the spores are shot, or that there is a definite reaction to light in this phenomenon.

#### BEHAVIOR OF THE FUNGUS IN ARTIFICIAL CULTURE

Six kinds of culture media were used: cornmeal, oatmeal, bean, prune, potato, and alfalfa-stem decoctions. 20 grams of agar were added to each

liter of fluid, and then the media were titrated and brought to 10 Fuller's scale. As the perithecia were found only on dead leaves, or at least in spots well decayed, and therefore heavily infected with bacteria and generally with saprophytic fungi, especially *Alternaria*, the dilution method of isolation was found inadequate. A simple method of isolation was substituted which worked perfectly. A part of a leaflet containing mature perithecia was suspended in the top of a petri dish containing medium, and the dish was then placed in a window exposed to early morning sunlight. In two hours there was an abundance of spores, which were marked by inverting the dish and dotting the glass directly over the spores, then they were transplanted to sterile plates of the media mentioned above.

These spores germinated within four to six hours, and by the next morning had developed considerable mycelium. After this rapid beginning the mycelium seemed to become checked, and afterwards growth was very slow on all the media.

Within four days from germination, perithecium-like structures appeared in profusion on the mycelium. On examination, these were found to be not perithecia but empty stromata. This observation was made in August, 1923, and repeated with the same results in March, 1924. Similar negative results were likewise obtained by Jones under similar conditions, but the latter did obtain some perithecia by placing his cultures outdoors in shady spots. Evidently the stimulus for the production of perithecia was lacking in my cultures.

After this failure, another method of obtaining spores under artificial conditions was attempted. On March 10 leaves containing typical *Pleospaerulina* spots were placed on filter paper, kept soaking wet, in the bottom of a moist chamber. The top was left off. This was placed in a well lighted room, but never exposed to direct sunlight. The temperature varied between 14° C. at night and 22° C. during the day.

Three days later, on examination an abundance of perithecia was found on all the leaves that were entirely dead. All of these contained asci with viable spores. From a macroscopical view the mycelium could be seen all over the leaf, and the perithecia were nearly superficial instead of being partially immersed in the substratum as is the case in the field. In this manner an excellent opportunity was afforded for studying the fungus and for obtaining a plentiful supply of spores for inoculation purposes.

After the failure to produce perithecia in cultures in the laboratory, an attempt was successfully made to grow them out of doors in the same manner in which Jones succeeded.

On March 7, large tubes, containing the agar decoctions of cornmeal, oatmeal, prune, bean, and potato respectively, were inoculated with single spores and placed outside the building. Black bodies formed on the mycelium in four days; however, they did not appear so large or in such abundance as the stromata in the cultures kept indoors. They were

examined each day with negative results until March 28, when, on the oatmeal medium, of twelve perithecia examined three were found to contain asci with mature spores.

The medium used in this case was the same as that used in the laboratory, and the only differences were due to being outdoors and being uncovered, the fungus thus having free access to the air. The changes from night to day, of course, were greater on the outside than in the building.

To determine further the factors for perithecial production, another experiment was devised. Alfalfa leaves were placed in petri dishes on wet filter paper and sterilized, then inoculated with single spores. Two such dishes were put in an incubator and kept at an even temperature of 21° C. Two were alternated, at first 3° C. for 12 hours, followed by 21° C. for 12 hours. Then two plates were placed out of doors on the north side of the building. The temperature out of doors varied from 7° to 24° C. between night and day.

After four days the three sets were found to contain black bodies on all the infected leaves. Examining them minutely, it was found that those kept at an even temperature produced only stromatal bodies without asci; those out of doors produced an abundance of fruiting perithecia; those kept indoors but subjected to alternations of temperature also produced spores. This result would suggest that the alternating temperature was the contributing factor out of doors.

#### RELATION OF A RED SPIDER SPOT TO THAT CAUSED ON ALFALFA BY *PLEOSPHAERULINA BRIOSIANA*

Both Jones and Melchers described the fungous spot on alfalfa as being 1 to 2 mm. in diameter, light gray to light brown with a dark-brown border, and scattered irregularly over the leaflet. The writer has repeatedly noticed in the field at certain times of the year that the majority of the leaves bear spots of this description; but it was also noticed that, whereas the old spots were of this nature, the young or fresh spots were distinctly of insect origin. Leaves with fresh spots were therefore marked in the field; others were taken to the laboratory and placed in moist chambers. In all instances these insect spots when old answered identically to the description heretofore given for those caused by *Pleospaerulina*.

The description of the insect spot when freshly made is as follows: with the naked eye, 1 to 2 mm. in diameter and appearing white from both sides of the leaflet; under the low power of the microscope, the under side was intact, no hairs or epidermal cells being disturbed, but on the upper side no hairs were found and the epidermal cells appeared disrupted and shrunken. In many free-hand sections the mesophyll was always seen to be intact, but the chlorophyll was bleached out. Insect excreta were always found on such leaves. The origin of these spots was verified by Dr. Krafka, zoologist at the University of Georgia.



These spots when mature took on the character of *Pleosphaerulina* spots; the following experiments were therefore made in an attempt to throw some light on the connection between the two.

(1) Fifty leaflets each containing many of the fresh spots of insect origin were bleached in alcohol by boiling for two minutes. Then they were examined under the low power. Dipping in eosin results in the spores and mycelium being stained in contrast with the leaf-green.

Of the fifty leaflets, thirty-two bore one to ten spores each, for the most part on the upper side. None of these spores had germinated to any extent. Only two leaves bore spores just beginning to put out germ tubes. The spores were scattered irregularly over the leaflet and seemed to bear no relation to the insect spots, and no mycelium could be seen in any of these spots.

(2) Fifty leaflets containing such spots both fresh and old, and some with no spores, were placed in a moist chamber on filter paper kept saturated with water. The cover was left off.

Four days later perithecia appeared on the leaflets that were the most decayed at the start. They did not appear on any definite spots, but were irregularly scattered over the whole leaflet in great profusion. At the end of nine days other leaflets, not so far decayed at the beginning, developed perithecia, making a total of thirty-seven out of the fifty becoming infected. On the 11th day, three leaves bearing no definite spots developed perithecia. This development was regular, no definite spots appearing.

(3) Fifty leaflets containing typical dead spots heretofore described as *Pleosphaerulina* spots were bleached and examined under the low power. All the leaflets had from three to twenty spots each, and some contained wide dead areas at the apex. These leaflets were taken at random from various parts of the field.

At least eighty percent of the spots were found to contain the mycelium coming directly from a spore, the latter occasionally being found directly over the spot, but in most instances the spore was at one side, sometimes a distance of one millimeter from the spot.

(4) To determine the cause of these spots, the insects were closely studied in the field and various ones were placed on control plants in the laboratory. It was found that both green aphids and red spider produced a puncture which left a small white spot, and these insects were found in great abundance all over the field. It was also found that the physiological disease known as alfalfa white spot, described by Stewart, French, and Wilson (12), was very common, and that the spots turned brown and took on the characters of the *Pleosphaerulina* spot.

From these observations it would seem that the point of entry of the fungus is the insect spot.

## PATHOGENICITY

Neither Melchers nor Jones proved *Pleosphaerulina briosiana* to be pathogenic on alfalfa, observing it only as coincident with a spotting of the leaves. Jones found it fruiting occasionally on petioles and rarely in the spots which he described as *Pleosphaerulina* spots. According to Jones:

These small, uniform spots, 1 to 2 mm. in diameter, have often been found scattered abundantly over the leaves of young, vigorous plants when the large, dead areas bearing perithecia were difficult to find. . . . When the younger leaves were decolorized in alcohol and acetic acid and examined under the microscope, a large dictyosporous spore was found in the center of nearly every spot, and usually the penetrating germ tube could be observed.

However, his attempts at artificial inoculation did not succeed. Therefore, to determine whether or not this fungus could be parasitic under any conditions, the following experiments were performed.

## INOCULATION AND INFECTION EXPERIMENTS

On February 27, a pot of approximately 75 to 100 plants grown under control conditions was sprayed with ascospores of *Pleosphaerulina briosiana* by suspending infected leaves over the plants and placing the whole pot under a bell jar. Afterwards leaves were pulled off and spores were found on all of them. Up to April 22, no sign of infection had been observed on any of these plants nor on the checks set up at the same time.

Next, a similar pot of alfalfa plants was placed under a bell jar. In this case the leaves bearing the fruiting fungus were placed on the soil in the pot, and in this way the spores could shoot up and so spray the plants. On May 1, no infection had appeared.

A third such pot of alfalfa was placed under a bell jar, after having fifty leaves scratched with a needle and perithecia suspended over them. No sign of infection had appeared on this pot, either on the leaves so injured or on uninjured leaves.

On April 12, a control pot was treated in the manner of the one described above, except that ascospores were placed on fifty leaflets that were injured and on fifty that were not injured, with a camel's-hair brush. These plants were growing thriftily, and the needle injury healed over and so no actual dead spots occurred on the leaves. On April 30, these plants were thoroughly examined. Spores were found on all the leaves on which they had been placed, and most of the spores had germinated. However, in no case had the mycelium entered the leaf and produced infection.

From these experiments it would seem that under no conditions can the germ tube of this fungus enter the live epidermis of the alfalfa plant, not even when the latter has been mechanically injured. Also, from the artificial culture experiments mentioned in another paragraph, it was proven that dead leaves or even dead spots on leaves can be easily infected, and further that the fungus will remain in the dead spot unless the rest of the leaflet should die.

On April 18, about fifty leaflets on a control pot were burned with a hot needle, and some were merely dotted finely with the needle, while others were burned over an area of several square millimeters. These leaflets were all inoculated from a pure culture, and fifty sound leaflets were also inoculated as a check.

After five days perithecia were produced in abundance on the large burned areas. However, there were no definite spots formed in these areas, and up to April 30, the fungus had not spread outside of the original burned areas. Where only small dots were made with the hot point of the needle, they became infected and a small spot resulted which resembled the *Pleosphaerulina* spot in the field. Up to May 1, no perithecia have appeared in these spots. The checks developed no fruiting bodies or signs of the fungus.


On April 20, leaves containing fresh spots made by aphids and also by red spiders were inoculated with spores. Ten days later the fungus had appeared in these injuries. The mycelial growth confined itself to these insect spots, but so changed their character as to make them appear as the *Pleosphaerulina* spots.

From all this evidence it would appear that *Pleosphaerulina briosiana* is not a parasite on alfalfa as has been thought, but is truly a saprophyte following an entry made by an insect or by the alfalfa white spot. The shape of the spot is also not caused by the fungus, because when the burned spot was large the fungus covered the whole, and when it was minute the mycelial growth was confined to the spot. Further, the leaf dies only after its resistance has been broken down by many insect attacks.

#### HOW THE FUNGUS SPREADS

*Pleosphaerulina* has been found on alfalfa in most of the old fields of this district as well as in others less than one year old. There are many difficulties involved in attempting to determine just where the spores come from. There are several possibilities. In the first place, locally the spores may be carried by the wind or by insects from one field to another. Then, when it appears for the first time in a field widely separate from any other field, there is the possibility that it is carried either as viable spores on the seed or as mycelium growing on the seed. Or it might be that the usual custom in this state of inoculating with *Bacillus radicicola* by using soil from another field brings along the spores in the *débris*. Then the infection may be carried by other plants than alfalfa.

In October, 1922, the Agronomy Department of the Agricultural College planted 40 test rows of alfalfa with different strains to every two rows. Some of these seeds were from Kansas and other points known to be infested with *Pleosphaerulina*. The inoculation for *Bacillus radicicola* was made from soil taken from a field containing this fungus. Near this plot alfalfa was growing in other plots and volunteer plants were in the bordering turnrows.



Nothing was observed on the plants until the following March, when they were about three inches high. At first the border rows were seen to be heavily infected with the typical *Pleosphaerulina* spots. Then gradually all the plants in the field developed it. It may have come from any of the sources enumerated or from all. However, the facts seemed to indicate that the bulk of the infection came from nearby plants.

In August, 1923, an attempt was made to determine this point definitely. 40 pots were planted to alfalfa and kept under control conditions in the greenhouse of the Botany Department of the University of Georgia. The pots and soil were all sterilized. In ten the seeds were treated by the same method successfully used by Hopkins (2) in treating seed of *Medicago arabica* for *Cercospora medicaginis* E. & E., and inoculated with pure cultures of *Bacillus radicolica*. In the next twenty the seeds were not treated, but other conditions were the same as in the first ten. In the last ten the seeds were treated as in the first, but the inoculation was from soil taken from a field known to be infected.

These plants grew to about three inches in height, and at that time the fungus had not appeared on any of them. Then, in consequence of my absence in September, they were allowed to die.

In January, 1924, the experiment was repeated and so far has yielded only negative results. So it can not be that this fungus is extensively carried with the seed, or with the *Bacillus radicolica* inoculum, as is true for *Cercospora medicaginis*.

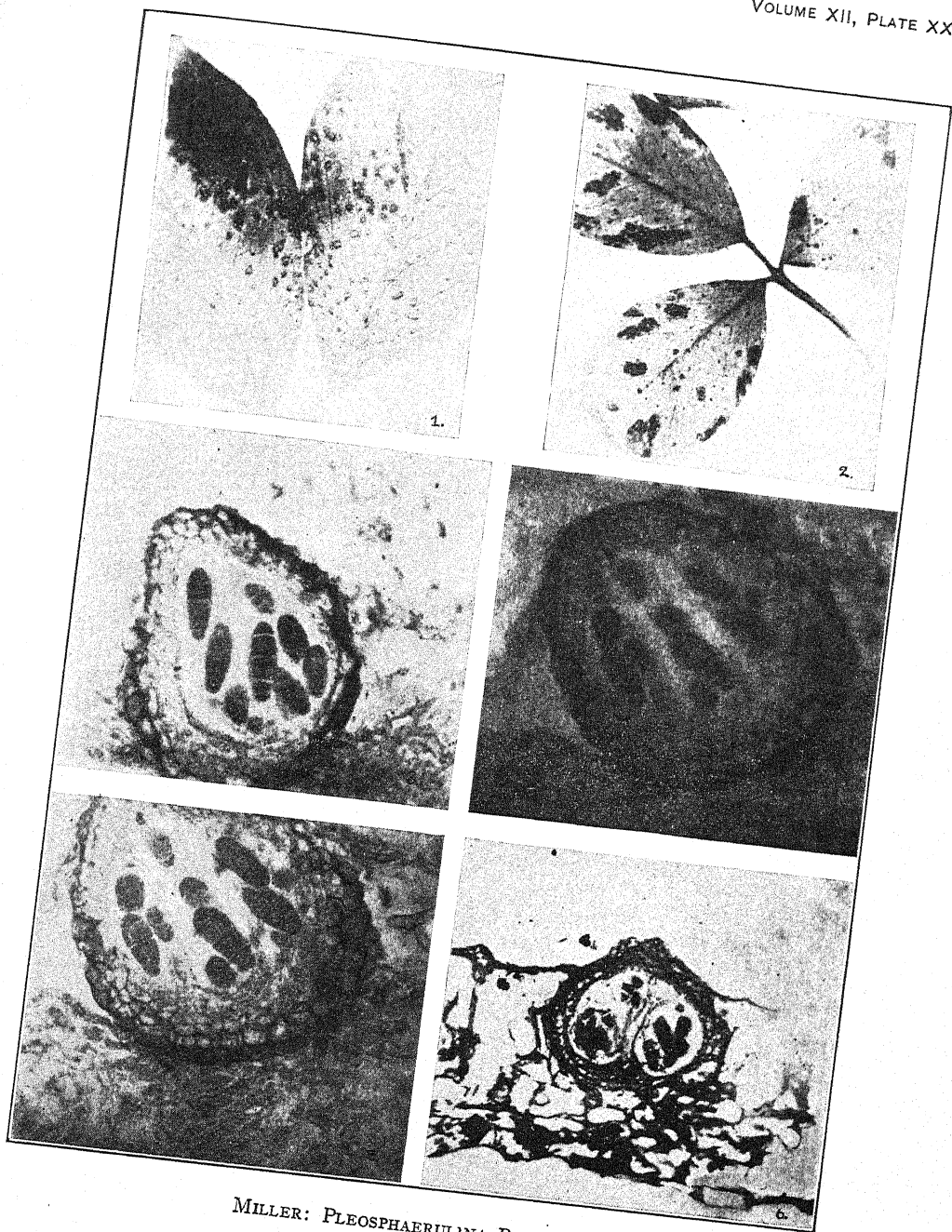
The possibility of its spreading from native plants of the same genus or related genera is very remote, because the writer has not been able to find it on any plant but alfalfa, and at present that is the only host on which it is known to occur.

As to the distance the spores can travel, there is a small patch of alfalfa on the university campus fully one mile from any large field, and there is another patch in another part of the city at least two miles from an infected field. Both of these plots have been here for many years, in fact long before *Pleosphaerulina* was discovered in Kansas. Neither patch has shown any signs of the presence of this fungus during the last three years in which the writer has observed them. Therefore it is probable that these spores do not travel very far from the seat of infection.

#### SUMMARY

1. The fungus collected here is identical in all respects with the original described by Pollacci except as to the color of the ascospores. The color of the spores of the other species of *Pleosphaerulina* should therefore be investigated more fully.

2. *Pleosphaerulina briosiana* Poll. is not a parasite on alfalfa. The spots accredited to it are made by an insect, and it follows saprophytically, confining itself to the spot, unless the entire resistance of the leaflet is broken down by many insect attacks.



MILLER: *PLEOSPHAERULINA BRIOSIANA*



3. The method of its spreading to new fields is not known. It spreads to young plants after a cutting by the forceful ejection of ascospores from perithecia on leaves lying on the ground.

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#### PLATE XXI

FIGS. 1, 2. Alfalfa leaves magnified to show the character of the *Pleosphaerulina* spot. Note the definiteness of the small spots and the irregularity of the larger dead areas. The fungus confines itself within the smaller spots unless the resistance of the leaf is entirely broken down, in which case it spreads to larger areas.

FIGS. 3-5. Photomicrographs of perithecial sections. These sections were stained in alum carmine for ten minutes and counterstained for ten seconds in methylene blue. This treatment gives good differentiation of blue-green spores and pink epiplasm and perithecial walls. Taken with Leitz objective 6 and ocular 12.

FIG. 6. Photomicrograph of a section of a perithecium. In this case no ocular was used, only Leitz objective 6. This section was stained with Delafield's haematoxylin and counterstained with eosin.

# CHROMOSOME CONSTRICTIONS AS DISTINGUISHING CHARACTERISTICS IN PLANTS

WM. RANDOLPH TAYLOR

(Received for publication May 26, 1924)

## INTRODUCTION

The tendency of studies on the form of chromosomes is frequently to demonstrate differences between the members of a given complex. The series of forms that may thus be established may be characteristic of a genus, of a species, or perhaps only of a race or of an individual. The more trivial the distinctions between the chromosomes, the less likely are these to hold good for major groups. While the information regarding these matters in animals is considerable, the whole topic has been in an unsatisfactory state for plants, and has only recently been subjected to critical reinvestigation. It has long been known that plant chromosomes differ in size in a given complex. Strasburger described such differences for *Funkia Sieboldiana* in 1883. Such cases deal simply with differences in appearance among the non-homologous chromosomes, but cases of differences between the members of a single pair are well known in animals and one case in plants would seem to exist in *Sphaerocarpus Donnellii* described by C. E. Allen. This apart from the pairing of unequal homologues which may be referable to hybrid origin. The third phase of this general topic deals with shape characters apart from mere size. For plants the features which have been noted may be roughly distinguished as follows: (1) Forms directly concerned with the position of attachment of the spindle fiber and correlated with a constriction at the zone of attachment. (2) Forms with constrictions unrelated to the fiber-attachment. (3) Comparatively small bodies attached to a chromosome by a cord of considerable length, the length of the cord being even equal to or greater than the diameter of the body itself. These bodies have been called "satellites." They do not seem in some cases to be well marked off from constrictions of the second class. Sometimes associated with a constriction is a lessening of the power to retain the stain, resulting in the constricted region being not only narrower but lighter in color than the rest of the chromosome. These features are quite independent of such disturbances as might produce fractures of chromosomes. Their obviousness may be increased by subjecting the tissue to certain agents such as chloral hydrate for a time before killing, as was shown by Sakamura and others. Sometimes such treatment demonstrates constrictions where they were not visible without it. However, there is much evidence to show that these constrictions represent normal features of



internal differentiation in the chromosome. They seem to be constant in their characteristic position on the chromosome and subject to no more distortion by the action of the fixing fluid than other features of the cell. However, they are easily obliterated by imperfect preservation, especially following slow penetration, the result of which is a longitudinal contraction of the chromosome and a return to an unbroken continuity. Consequently, failure to demonstrate them can not be accepted as positive evidence of their absence.

These features of chromosome construction may be suggested as having possibilities of usefulness in correlation with genetical studies. In order that a correct estimation of their importance and limitations may be reached, a very considerably greater series of plants must be studied than has yet been reported, and the present paper has been prepared to indicate certain features which must be considered in such studies, rather than as an exhaustive analysis of each of the plants discussed. The writer has presented the literature dealing with constrictions in plant chromosomes in earlier contributions, to which reference may be made (12, 13, 14).

One of the important questions arising is, how far down the scale of descending chromosome size the constrictions may be demonstrated; whether in fact they are a feature whose obviousness is directly related to chromosome size. It seems that most genetical work is being done on plants with chromosomes of quite moderate size, and the features described for the large chromosomes of *Gasteria* might be thought not to hold good in these other cases.

#### MATERIAL AND OBSERVATIONS

##### *Pitcairnia xanthocalyx* Mart.

Smears made of the developing microspore mother cells of this plant showed the haploid chromosome number to be 25. The chromosomes are small and elliptical or nearly spherical (Pl. XXII, figs. 1-5). They did not show constrictions in the maturation divisions.

##### *Aechmea conspicuiarmata* Baker?

Sections of root tips from small plants showed the diploid number to be probably 50 chromosomes (Pl. XXII, figs. 6, 7). The only report with which the writer is familiar giving a count in the Bromeliaceae is by Billings (2) for *Tillandsia usneoides* where  $N = 16$  was determined for developing microspore mother cells. In *Aechmea* the chromosomes are small and somewhat elliptical with pointed ends. In general they move to the poles in anaphase with their long axes at right angles to the direction of motion. Consequently one would expect to find the fiber-attachment near the middle of the chromosome, but no constriction is visible. In view of the size of the chromosomes it is not likely that one could be demonstrated if it really exists.

### *Cleome spinosa* L.

Sections of radicles from germinated seeds gave good plates which seemed to indicate a  $2N$  count of 38 chromosomes (Pl. XXII, figs. 8, 9). The chromosomes were small, tapering to the ends, more or less curved, and, like those of *Aechmea*, passed to the poles extended in a transverse plane. No constrictions were visible in these chromosomes. Tischler (15) gives the probable count for *Cleome paradoxa* as  $N = 16$ .

### *Coix Lachryma-Jobi* L.

This genus has been reported upon by Kuwada (5, 6), as having a chromosome number of  $2N = 20$ , which is sustained by the present study. The chromosomes are fairly small, but stand beautifully separate at metaphase, show some indications of size differences, taper to blunt ends, and are usually bent at the middle with greater or less indications of a median constriction (Pl. XXII, figs. 10, 12, 14). The constriction probably is normally present in all, but the preservation was not quite adequate to give a uniform picture. In anaphase (Pl. XXII, figs. 11, 13) the chromosomes pass to the poles bent near the middle by a median fiber-attachment, but no constriction was surely recognized, probably because of the exceeding smallness of the chromosomes.

### *Fagopyrum esculentum* Moench.

This plant has been reported by Stevens (10) to have a chromosome number of  $N = 8$ , which statement is sustained by the diploid count of 16 made by the present writer in the radicles. The chromosomes are fairly small, somewhat enlarged toward the blunt tips, and more slender toward the middle where they are bent and usually show a fiber-attachment constriction (Pl. XXII, figs. 15, 17, 19). During anaphase they become so bent that the arms may be nearly parallel and the constriction can usually be recognized.

### *Eichhornia speciosa* Kunth

Smith (9) reports for *Eichhornia* and *Pontederia* that there are 14 to 16 chromosomes in the haploid condition. Further he states that the chromosomes never become "V"-shaped, by which he probably means that the fiber-attachment is always essentially terminal. The chromosomes were mainly studied in microspore-formation. The present writer has studied root-tip sections of *Eichhornia speciosa* (*E. crassipes*), which showed probably 32 chromosomes as the diploid condition. These were rather small, yet it could clearly be seen that they were to be separated into two classes, one with the fiber-attachment constriction near the middle, the other class with it asymmetrical. It is obvious on close examination that there are size distinctions in each group and also that of those with the asymmetrical

fiber-attachment part had the closely subterminal type described for other plants, while some had a rather longer interval between the tip and the fiber-attachment. However, the chromosomes were too small to make it advisable to attempt a complete classification, especially in the absence of good maturation-division figures.

### *Yucca filamentosa* L.

Several writers have mentioned the chromosome size difference existing in the members of this genus. Müller (7), studying root-tip material of *Y. aloifolia*, *Y. guatemalensis*, and *Y. draconis*, found that the diploid number is about 54-56 small chromosomes and 10 large ones. Wóycicki (16) found for *Y. recurva* that there are about 44 small chromosomes and 10 larger ones, Bonnet (3) for *Y. gloriosa* gave the number as at least  $N = 25$  and figures the size differences, but his figures are exceptionally unsatisfactory, indicating much clumping of the chromosomes. Radicles of *Y. filamentosa* gave good preparations showing the diploid number of 10 large chromosomes and a number of small ones. None of the latter showed any evidence of fiber-attachment constriction, but such a constriction could be seen in some cases in the larger ones and was closely subterminal (Pl. XXII, figs. 22, 23).

### *Crepis capillaris* (L.) Wallr. and *C. setosa* Hall

A number of workers have studied species of *Crepis*, making this one of the best known genera of the Compositae cytologically. Their work is summarized by Tischler (15). Recently *C. capillaris* (*C. virens*) has been studied by Navašin (8), but the writer has not been able to secure a copy



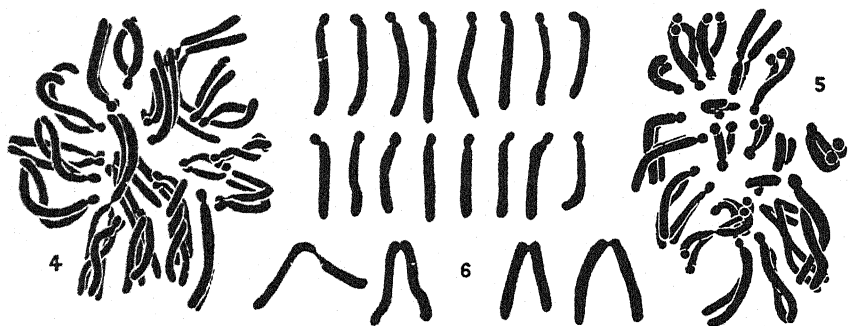
TEXT FIGS. 1, 2. *Crepis setosa*, metaphase, root-tip mitosis, showing fiber-attachment constrictions and satellites. FIG. 3, *C. capillaris*, ditto.

of the paper. Miss Mann, Babcock, and Collins have reported on a long series of chromosome counts and related genetical studies (1, 4), but the figures and descriptions of the chromosomes which they supply do not give a very extensive idea of the form-variety in the different species. To a certain extent chromosome size differences are visible, but the fiber-attachment position can not be determined. The chrom-acetic fixation used is not favorable for such a study. The investigation of individuality characters

of this type and of satellites would seem to be extremely interesting and highly important and practicable here. The writer has been able to examine root-tip cells of *C. capillaris* and *C. setosa* through the kindness of Dr. R. T. Hance, who had these plants in culture. *C. setosa* has four pairs, all different (text figs. 1, 2). The fiber-attachment appears to be comparatively near the end in all, but with some differences evident. One pair is provided with splendidly developed satellites (see also 1, page 7, fig. 33 A). *C. capillaris* has three pairs, also all different (text fig. 3). The largest has the attachment constriction removed from the end by a considerable distance, the medium-sized pair has it closely subterminal. The smallest pair bears satellites at the fiber-attachment end, but the attachment constriction was not determined because of somewhat inferior preservation.

### *Sagittaria montevidensis* Cham. & Schlecht.

This plant does not seem to have been studied in detail cytologically. In connection with studies on dioecious plants Miss Sykes (11) reports that with other plants *S. montevidensis* does not show chromosome differences related to sex. The count is not given. The chromosomes in the root-tip cells are so long and twisted that counts here are difficult, but the diploid number appears to the writer to be probably 20. The roots of this plant fix well, and the cells give splendid examples of the fiber-attach-



TEXT FIGS. 4, 5. *Sagittaria montevidensis*. Metaphase, root-tip mitosis. FIG. 6. Ditto, anaphase chromosomes from root-tip cells showing the two types of fiber-attachment constriction.

ment constrictions of the closely subterminal type. Certainly one pair and probably two have the fiber-attachment median in position. This is hard to determine in such complicated metaphases, but when anaphase groups are examined and the number of long arms of chromosomes counted the number seems to be 24, confirming to some extent the view offered.

*Aloë saponaria* Haw.

This plant has been discussed in a previous contribution (13), and is introduced here mainly to give for comparative purposes a type with rather large chromosomes (Plate XXII, fig. 24). Of the seven pairs, three are quite small with subterminal attachment, three large ones have the fiber-attachment rather distant from the upper end, and the remaining pair have their fiber-attachments subterminal and bear satellites at the free ends (not showing in the figure).

## SUMMARY

The maturation divisions of the microspore mother cells of *Pitcairnia xanthocalyx* showed no evidence of spindle-fiber-attachment constrictions in the rather small chromosomes. Root-tip mitoses of *Aechmea conspicui-armata*, *Cleome spinosa*, and *Yucca filamentosa* (smaller chromosomes) showed none in the very small chromosomes concerned. Root-tip mitoses of *Fagopyrum esculentum*, *Coix Lachryma-Jobi*, and *Yucca filamentosa* (larger chromosomes) showed some indications of these constrictions in the rather small chromosomes, while in chromosomes of the same general size in *Eichhornia speciosa* they were quite distinct. Constrictions for fiber-attachment were clearly visible in the moderately large chromosomes of *Sagittaria montevidensis*, *Crepis capillaris*, and *C. setosa*, with satellites also present in the last two cases, and very distinct also in the large chromosomes of *Aloë saponaria*, which possess similar satellites.

The writer wishes gratefully to acknowledge the assistance of Miss E. G. Stiffler in preparing for study the material with which this paper is concerned.

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### DESCRIPTION OF PLATE XXII

All figures in this paper are reproduced to give a magnification of approximately 2000 diameters.

FIGS. 1, 5. *Pitcairnia xanthocalyx*, first maturation-division metaphase plates, microspore mother cell.

FIGS. 2, 4. Ditto, first maturation-division anaphase plates from the same microspore mother cell.

FIG. 3. Ditto, two second maturation-division metaphase plates from sister cells in microspore-development.

FIGS. 6, 7. *Aechmea conspicuiarmata*, metaphase plates of root-tip mitoses.

FIGS. 8, 9. *Cleome spinosa*, metaphase plates of root-tip mitoses.

FIGS. 10, 12, 14. *Coix Lachryma-Jobi*, metaphase plates of root-tip mitoses.

FIGS. 11, 13. Ditto, anaphase chromosomes of root-tip mitoses.

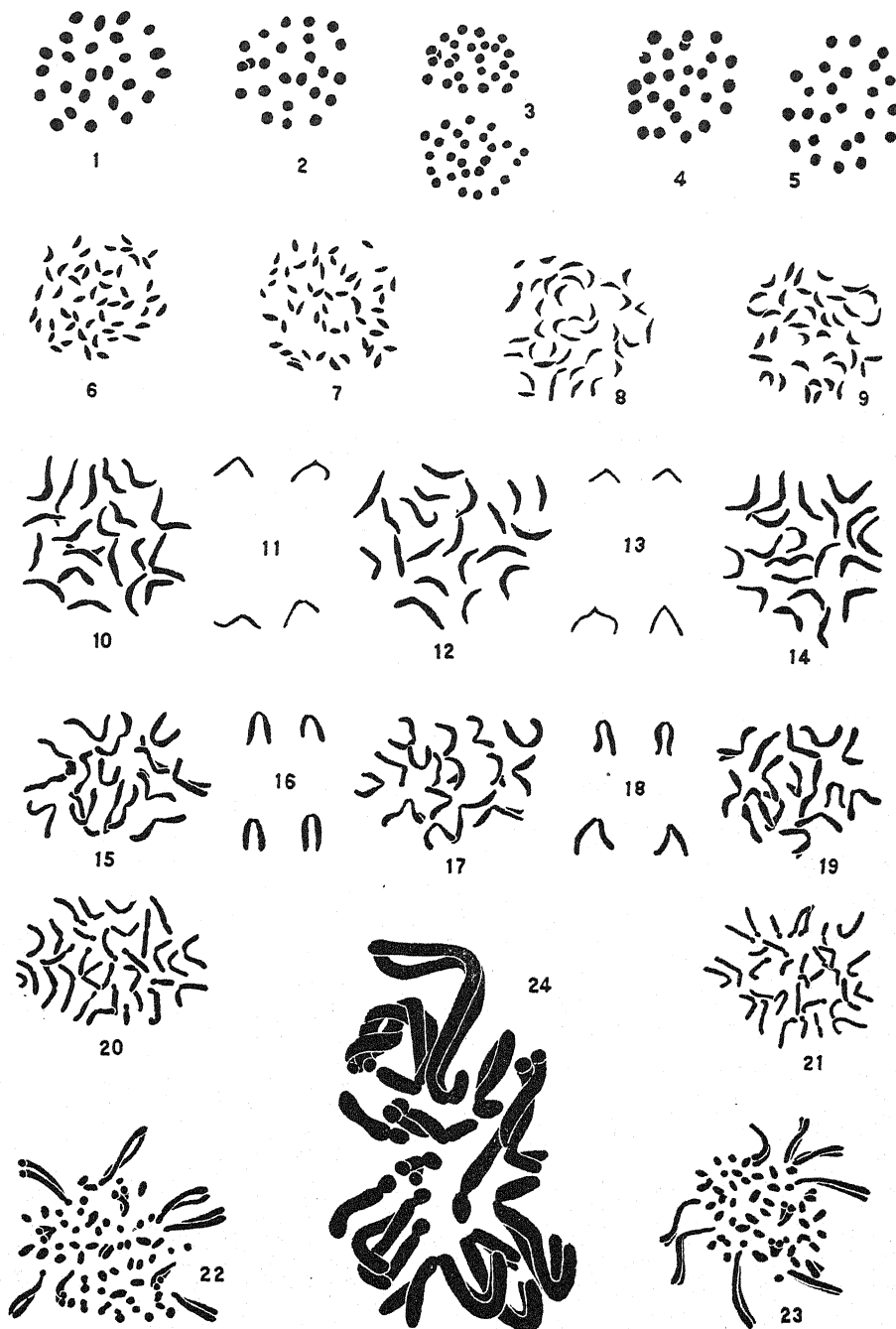
FIGS. 15, 17, 19. *Fagopyrum esculentum*, metaphase plates of root-tip mitoses.

FIGS. 16, 18. Ditto, anaphase chromosomes of root-tip mitoses.

FIGS. 20, 21. *Eichhornia speciosa*, metaphase plates of root-tip mitoses.

FIGS. 22, 23. *Yucca filamentosa*, metaphase plates of root-tip mitoses.

FIG. 24. *Aloë saponaria*, metaphase plate of root-tip mitosis.







## VARIATIONS IN A MEMBER OF THE GENUS *FUSARIUM* GROWN IN CULTURE FOR A PERIOD OF FIVE YEARS

WALTER H. BURKHOLDER

(Received for publication May 31, 1924)

Before the pathogene *Fusarium martii phaseoli* Burkholder was described, three cultures of *F. martii* Ap. et Wr. were obtained from various sources for comparison. One culture was from Dr. C. D. Sherbakoff, the second from the Wollenweber collection in the Bureau of Plant Industry, and the third from Dr. Johanna Westerdijk at the Centralstelle für Pilzkulturen. That there were differences among the sub-cultures of these three strains of *F. martii* was observed, and in the article describing the form *F. martii phaseoli* (2) it was stated that "the spore measurements made from the culture obtained from the Wollenweber collection varied considerably from the measurements given by Appel and Wollenweber, and by Sherbakoff." These variations were considered to be due probably to the effect of the different lengths of time the fungi had been in culture, to various culture media used before they were received, to different temperatures to which they had been submitted, or to other similar environmental factors. That these variations induced by the environment had become sufficiently established to be retained to at least a certain degree when the three strains were placed under similar conditions was thought probable. Appel and Wollenweber (1) recognized a change in the morphology and physiology of species of the genus *Fusarium* which had been grown for a long time on artificial media, and they designated three stages in the life history of such a fungus, namely *Ankultur*, *Normkultur*, and *Abkultur*. The culture referred to above from the Wollenweber collection had presumably passed the "Normkultur" and entered the "Abkultur" stage. Sherbakoff (6) also mentions this latter stage in his work on the potato Fusaria, but states that the only change he has observed is the loss of color.

Since 1917, when the observations and spore measurements were made for the description of *F. martii phaseoli*, this fungus has been kept growing in pure culture. During this period, and especially during the last two or three years, the morphology (at least the gross characters) of the organism has changed to such an extent that it might be described as a new species. It has passed over into the "Abkultur" stage of Wollenweber. When grown on various media, including agars and sterilized vegetables, the fungus does not resume its original characters. In this article the nature and permanency of these variations, both morphological and physiological, that have been brought about through continuous cultivation are dealt with.

The strain of the fungus under consideration was isolated from a diseased

bean root during the summer of 1916. Spore-dilution plates were prepared from the original isolation, and a transfer was made from what was considered after examination under the microscope to be a single-spore colony. This single-spore culture then was used for the description of this fungus. The conidial measurements and the various descriptions, together with the drawings appearing in Cornell Memoir 26 were made during the summer months of 1917. The pathogene therefore had been in culture at least a year before it was described. Since then, this strain has been kept growing in the laboratory at the ordinary room temperatures. Two different media only have been used. These media were sterilized green bean pods, and the ordinary stock-room potato agar.<sup>1</sup>

In March 1922 a number of bean plants were inoculated with the pathogene, and upon examination a month later it was found that 100 percent infection had taken place. Isolations were made from one of these infected plants, and a fungus was obtained which in gross appearance agreed with the original description of *F. martii phaseoli*. Excellent infection was obtained on bean roots with this fungus, and isolations from such roots gave a third fungus similar in appearance. Just what change, however, had taken place as to spore type and size in the old culture, and what effect the return to the host plant had on the microscopic characters of the pathogene were not known. With this problem in mind the following experiments were conducted. It was planned to grow the original strain and the two cultures obtained from reisolations on various media, and to make conidial measurements at the same ages as those used in describing the form species originally. In preparing the original description (2), the method employed by Sherbakoff (6) was followed. Also, the media used and the age of the individual cultures at the time of conidial measurements were similar to those in his description of *F. martii*. It was further planned to grow the cultures during the summer months, since it was at this time of year that the original description had been made. In the remainder of this article the three cultures used will be referred to by number for convenience. The original, six-year-old culture will be designated as strain I, the reisolation of strain I as strain II, and the reisolation of strain II as strain III. The various media used were potato agar containing one percent glucose, slightly acidified hard potato agar, sterilized green bean pods, red raspberry canes, and potato plugs. The formulae for the two agars are the same as used by Sherbakoff (6). Before transferring the three strains to the various media, inoculation experiments were conducted with them to make sure they were all strains of *F. martii phaseoli*. Infection took

<sup>1</sup> Potato tubers (peeled).....	200 g.
Glucose.....	10 g.
Sodium chlorid .....	5 g.
Shredded agar.....	18 g.
Water.....	1,000 g.

place in all cases, with the exception of the checks. An observation on these infections, however, proved to be of importance. It appeared by examination of the roots that the injury caused by strain III was more severe than those caused by strains I and II. It seemed probable that the pathogene during its six years of cultivation had lost some of its virulence. Experiments conducted to determine this point are reported upon later in this article.

#### CHANGES IN MORPHOLOGY

The following experiments were conducted during the summer of 1922. Ten tubes of each of the media mentioned above were planted with the three strains, to determine what variation had taken place since the original description was made. On most media there was a distinct difference in gross appearance between strain I, and strains II and III. In nearly all cases strain I produced a white, fluffy growth due to an abundance of aërial mycelium. The aërial mycelium was rather scanty in strains II and III, and the cultures were characterized by a decided coloring. These colors varied through the blues and greens, and were more intense in strain III. Great slimy masses of spores borne in pseudopionnotes also occurred only in the strains II and III. For making the measurements of conidia of the various types, one hundred conidia were selected at random. Types that were known to occur in a culture, but were not observed when measurements were made, were marked *rare*. It was considered that such types were present in less than one percent. These measurements are listed in the manner employed by Sherbakoff (6), and the original descriptions of *F. martii phaseoli* by the writer (2) are inserted for convenience.

There are several important variations that should be mentioned which are not brought out in the measurement of conidium types, and which should be considered before proceeding to the following descriptions. The production of macroconidia in strain I was very meager, and three and sometimes four cultures were searched to obtain a sufficient number to measure. An exception to this, however, was on the sterilized raspberry canes where the fungus from the old culture produced a fair-sized number of spores. Many of the conidia produced by this strain were abnormal in shape—that is, they were not of the true Martiella type, and since they varied greatly among themselves it was impossible to place them in any of the established sections of this genus. In strains II and III the macroconidia were normal, very uniform, and were borne in abundance in pseudopionnotes. On the other hand, strain I bore a considerable number of microconidia. This type of spore was rarely found in cultures of strains II and III. The measurements of the conidia on different media are as follows:

On bean plugs, cultures eighty-three days old.

Original description; conidia taken from pseudopionnotes:

Conidia: 3-septate, 38 percent,  $41.4 \times 4.9(37.7-46.5 \times 3.9-5.8) \mu$ ;

4-septate, 62 percent,  $47 \times 5(40.3-53.3 \times 4.5-5.8) \mu$ ;

5-septate, rare.

Strain I; conidia taken from aërial mycelium:

Conidia: 3-septate, 67 percent,  $43.1 \times 5.1(31.2-54.6 \times 4.8-5.8) \mu$ ;  
 4-septate, 31 percent,  $48.5 \times 5.2(42.9-58.5 \times 4.8-5.8) \mu$ ;  
 5-septate, 2 percent,  $50.7 \times 5.8 \mu$ .

Strain II; conidia taken from pseudopionnotes:

Conidia: 3-septate, 64 percent,  $44.6 \times 5.2(35.1-50.7 \times 4.8-5.8) \mu$ ;  
 4-septate, 35 percent,  $48.3 \times 5.3(42.9-54.6 \times 4.8-5.8) \mu$ ;  
 5-septate, 1 percent,  $54.6 \times 5.8 \mu$ .

Strain III; conidia taken from pseudopionnotes:

Conidia: 3-septate, 62 percent,  $46.6 \times 4.9(39-54.6 \times 3.9-5.8) \mu$ ;  
 4-septate, 36 percent,  $48.5 \times 4.9(42.9-54.6 \times 4.8-5.8) \mu$ ;  
 5-septate, 2 percent,  $48.7 \times 4.8(46.8-50.7 \times 4.8) \mu$ .

Raspberry-cane plugs, cultures eighty days old.

Original description; conidia taken from sporodochium:

Conidia: 2-septate, rare;  
 3-septate, 66 percent,  $41.4 \times 5(32-48 \times 4-5.8) \mu$ ;  
 4-septate, 34 percent,  $49.3 \times 5(44-56 \times 4-5.8) \mu$ .

Strain I; conidia taken from sporodochium:

Conidia: 3-septate, 42 percent,  $46.9 \times 5.3(39-58.5 \times 4.8-5.8) \mu$ ;  
 4-septate, 58 percent,  $54 \times 5.4(46.8-58.5 \times 4.8-5.8) \mu$ .

Strain II; conidia taken from sporodochium:

Conidia: 3-septate, 61 percent,  $48 \times 5.4(39-54.6 \times 4.8-6.8) \mu$ ;  
 4-septate, 39 percent,  $53.9 \times 5.6(46.8-58.5 \times 4.8-5.8) \mu$ .

Strain III; conidia taken from sporodochium:

Conidia: 3-septate, 51 percent,  $48.1 \times 5.5(42.9-54.6 \times 4.8-5.8) \mu$ ;  
 4-septate, 49 percent,  $53 \times 5.7(42.9-62.4 \times 4.8-6.8) \mu$ .

\* Potato agar containing one percent glucose, cultures 22 days old.

Original description; conidia from pseudopionnotes:

Conidia: 2-septate, 3 percent,  $37.7 \times 4.4(36-40 \times 4-5.3) \mu$ ;  
 3-septate, 83 percent,  $43.5 \times 5.2(33.3-56 \times 4-6.6) \mu$ ;  
 4-septate, 14 percent,  $52.3 \times 5.4(42.6-60 \times 4.6-6.6) \mu$ .

Strain I; conidia from aërial mycelium:

Conidia: 1-septate, 5 percent,  $31.8 \times 4.8(27.3-35.1 \times 3.9-5.8) \mu$ ;  
 2-septate, 3 percent,  $31.2 \times 4.5(27.3-35.1 \times 3.9-4.8) \mu$ ;  
 3-septate, 92 percent,  $43.7 \times 5.2(31.2-58.5 \times 3.9-7.8) \mu$ .

Strain II; conidia from pseudopionnotes:

Conidia: 3-septate, 45 percent,  $48.5 \times 5.3(35.1-58.5 \times 3.9-6.8) \mu$ ;  
 4-septate, 55 percent,  $52.2 \times 5.7(46.8-58.5 \times 3.9-6.8) \mu$ .

Strain III; conidia from pseudopionnotes:

Conidia: 1-septate, 1 percent,  $27.3 \times 5.8$ ;  
 2-septate, no count;  
 3-septate, 59 percent,  $46.8 \times 5.3(39-54.6 \times 3.9-6.8) \mu$ ;  
 4-septate, 40 percent,  $52.2 \times 5.7(46.8-54.6 \times 4.8-6.8) \mu$ .

Slightly acidified hard potato agar, cultures 18 days old.

Original description; conidia from pseudopionnotes (culture 11 days old):

Conidia: 3-septate, 24 percent,  $48.7 \times 5.3(35.3-53.3 \times 4.6-6.6) \mu$ ;  
 4-septate, 76 percent,  $52.9 \times 5.5(46.6-56 \times 4.6-8) \mu$ ;  
 5-septate, rare.

Strain I; conidia from aërial mycelium:

Conidia: 1-septate, 7 percent,  $28.4 \times 4(23.4-31.3 \times 4) \mu$ ;  
 2-septate, 8 percent,  $32.1 \times 4.5(25.3-42.9 \times 3.9-5.8) \mu$ ;  
 3-septate, 84 percent,  $36.2 \times 5(27.3-42.9 \times 3.9-7.8) \mu$ .

Strain II; conidia from pseudopionnotes:

Conidia: 2-septate, 1 percent,  $31.2 \times 4.8 \mu$ ;  
3-septate, 28 percent,  $51.8 \times 6.08(42.9-58.5 \times 4.8-7.8) \mu$ ;  
4-septate, 70 percent,  $56.1 \times 6.1(50.7-62.4 \times 4.8-7.8) \mu$ ;  
5-septate, 1 percent,  $58.5 \times 6.8 \mu$ .

Strain III; conidia from pseudopionnotes:

Conidia: 2-septate, rare;  
3-septate, 35 percent,  $49.25 \times 5.46(35.1-58.5 \times 3.9-6.8) \mu$ ;  
4-septate, 65 percent,  $54.1 \times 5.7(46.8-58.5 \times 4.8-7.8) \mu$ .

On potato plug, cultures eighty-three days old.

Original description; conidia taken from pseudopionnotes:

Conidia: 2-septate, rare;  
3-septate, 76 percent,  $43.5 \times 5.2(36-48 \times 4-8) \mu$ ;  
4-septate, 24 percent,  $46 \times 5.5(42-49.3 \times 4.6-6.6) \mu$ .

Strain I; conidia taken from aerial mycelium:

Conidia: 1-septate, 9 percent,  $24.7 \times 5(23.4-27.3 \times 4.8-5.8) \mu$ ;  
2-septate, 2 percent,  $27.3 \times 5.8 \mu$ ;  
3-septate, 69 percent,  $40.6 \times 5.2(27.3-54.6 \times 4.8-5.8) \mu$ ;  
4-septate, 20 percent,  $45.6 \times 5.5(39-50.7 \times 4.8-5.8) \mu$ .

Strain II; conidia taken from pseudopionnotes:

Conidia: 3-septate, 86 percent,  $39.3 \times 5.1(27.3-50.7 \times 4.8-5.8) \mu$ ;  
4-septate, 14 percent,  $45.1 \times 5.1(39-46.8 \times 4.8-5.4) \mu$ .

Strain III; conidia taken from pseudopionnotes:

Conidia: 3-septate, 46 percent,  $40.5 \times 5.6(27.3-50.7 \times 4.8-5.8) \mu$ ;  
4-septate, 54 percent,  $47.4 \times 5.6(39-54.6 \times 4.8-5.8) \mu$ .

In looking over the measurements of the conidia, one will observe that the percentage of spore types in strain I varies most from the original, throughout all the media used. This character at first might appear to be of little taxonomic value, but when it is observed that a close approximation to the original is again obtained in many cases when the fungus is allowed to infect a bean plant and is then reisolated, the character seems worthy of a little consideration. On bean plugs and red raspberry canes, further appreciable microscopic changes in strain I from the original description are lacking. On the agars and on potato plugs, however, there are a considerable number of variations in other characters. In strain I on these three media there are notably changes in size of conidium types, loss of conidium types, and the appearance of abnormal types or of types not noted in the original description. On the two agars, 4-septate conidia are entirely lacking; on the acidified potato agar the 3-septate spores are  $12 \mu$  shorter than the original, which difference seems to be of some significance; and the percentage of abnormal types on the potato agar plus 1 percent glucose, the acidified potato agar, and the potato plugs are 5, 15, and 11 percent respectively. In all three cases the abnormal types were conidia having fewer septa than any found in the original description.

One may observe in examining the sizes of the conidium types that in strains I, II, and III they were slightly larger than given in the original descriptions. Most measurements show them to be 2 and  $3 \mu$  longer and

up to  $1\ \mu$  wider. These differences, however, are rather small, and since they are consistent throughout are probably due to the technique of measuring. They are disregarded here as being insignificant.

### CHANGES IN PHYSIOLOGY

To determine whether or not there is a difference in the virulence of the three strains of the pathogene, as appeared to be the case from the observation mentioned above, certain inoculation experiments were conducted in a similar manner to those described by the writer (3) in his studies in soil moisture. In brief, two plants were grown in each culture jar. Eight jars each were inoculated with strains I, II, and III of the pathogene, and eight remained uninoculated as a check. The soil moisture and other environmental factors were held as nearly uniform as possible in all the jars. The experiments were set up the middle of July, and the seeds were harvested in October. The results of these experiments are to be found in table 1.

TABLE 1. *The Result of Inoculation Experiments with the Three Strains of F. martii phaseoli*

Pot Number	Weight of Seeds (grams)		Infection	
Check				
	Plant 1	Plant 2	Plant 1	Plant 2
1.....	3.56	4.90	light	none
2.....	5.46	4.89	none	none
3.....	4.67	4.15	none	none
4.....	3.72	4.56	none	none
5.....	4.43	5.95	none	none
6.....	5.78	4.88	none	none
7.....	4.73	4.83	none	very slight
8.....	5.18	4.11	none	very slight
Inoculated with Strain I				
9.....	3.86	3.90	fair	good
10.....	4.37	2.56	fair	good
11.....	4.17	4.28	fair	fair
12.....	3.34	2.88	good	good
13.....	4.60	1.49	fair	severe (canker)
14.....	3.81	2.94	light	good
15.....	4.89	3.11	fair	fair
16.....	4.44	6.06	fair	light
Inoculated with Strain II				
17.....	1.71	3.62	severe	severe
18.....	1.77	4.76	good	none
19.....	3.72	4.79	good	good
20.....	2.39	4.23	severe	good
21.....	4.86	2.64	good	severe
22.....	4.87	4.13	fair	fair
23.....	4.47	3.50	fair	severe
24.....	4.32	4.91	fair	fair

*Inoculated with Strain III*

25.....	4.59	1.89	severe	severe
26.....	2.54	.91	severe	severe
27.....	2.50	2.98	severe	severe
28.....	.33	2.51	severe	severe
29.....	2.77	3.23	severe	severe
30.....	5.02	.79	good	severe
31.....	3.47	3.06	severe	severe
32.....	0.00	7.10	severe	fair

The weights of the seeds from the individual plants appear fairly uniform throughout each set, with the exception of several cases that are partially explained by the degree of infection. For this reason, and also because the percentages of differences of the infected sets from the check are sufficiently close or far enough removed from each other to be significant, it appears safe to treat each group as an individual unit. The weight of the seeds in the check group is 75.80 grams, while the weight of the seeds in the two sets infected by strain I and strain II are 60.70 grams and 60.69 grams respectively. Here we have a reduction in yield of 20 percent. Allowing the six-year-old strain to grow on its host plant for a month appears not to have had any effect in restoring its lost virulence.

The weight of the seeds from plants infected by strain III, however, was only 43.69 grams, or a reduction of 42 percent in yield from the check. This strain, it should be recalled, was recovered from a bean plant infected by strain II. It appears, therefore, that strain I requires more time than one month on its host plant to restore its virulence. It is probable that the full virulence of the fungus has not been entirely recovered in strain III, since infection experiments conducted in 1918 and reported by the writer (2) show a reduction in yield of 50 percent or more by the pathogene under favorable conditions. On the other hand, the conditions surrounding the 1918 experiments and those described here are sufficiently diverse to account for a difference in reduction of 10 percent.

## CONCLUSIONS

From the above-described experiments it may be seen that the morphology and the physiology of the species of *Fusarium* under consideration were considerably changed during the relatively short period of five years in culture. These changes progressed far enough to make identification of the fungus difficult, and only a return to its original host plant brought back to any extent its former characters. That these changes also take place in nature seems reasonable to expect, and the fungus, being a hemiparasite, might be allowed to proceed for a great many years without a return to the bean plant. The question then naturally arises whether these variations might not eventually become permanent. The fact that there is a large number of closely related species and varieties in the genus

Fusarium would lead to this belief. For instance, we have in the *martii* group the original species *F. martii* Ap. et. Wr., two varieties listed by Sherbakoff (6), *F. martii* Ap. et. Wr. var. *minus* Sherb. and *F. martii* Ap. et Wr. var. *viride* Sherb., *F. martii* Ap. et Wr. var. *pisi* F. R. Jones,<sup>2</sup> a variety recently described as parasitic on the roots of peas (5); the form species *F. martii phaseoli* Burkholder, dealt with in this article; and *F. eumartii* Carpenter (4) which causes a disease of potatoes. No doubt other forms also belong to this group. All these fungi vary from each other by single or minor characters, and it is probable they all had a common origin. That some plan might be adopted to hold such groups together in a taxonomic way to show the relationship as it is found in this case, would seem highly desirable.

A further point that arose in connection with this work has to do with the decline in virulence of the pathogene. It has been frequently observed in the bean sections of New York state that where beans follow beans in short rotation the disease is more severe than where very long rotations are practiced. This was considered to be due to the fungus being starved out of the soil in the latter case, since no other host plant has been found for it. Thus there would be a diminished infestation. Nevertheless it is known that the pathogene grows very readily in a saprophytic state upon decaying bean stems and might remain in the soil for a great many years. Furthermore, in fields where the disease is doing little damage practically all plants are infected, but the disease is not progressing rapidly in the plants. In the light of the experiments recorded in this article, a further explanation might be offered, if not to supplant, at least to supplement, the diminished-infestation theory. Since a loss of approximately 50 percent in virulence occurred in *F. martii phaseoli* in culture during five years, a similar occurrence might take place in the soil in the absence of the bean plant. If this were true, the severity of infection would be greatly reduced where very long rotations were practiced. Whether, however, the saprophytic state in a test tube is comparable to that on decaying vegetation is a question that should be taken into account.

#### SUMMARY

1. Morphological and physiological changes take place when *F. martii phaseoli* is grown in pure culture for a considerable length of time.
2. There is a gradual but pronounced change in the macroscopic characters of the fungus. When recently isolated the pathogene produces on most media a blue-green slimy growth. At the end of six years the growth is white and fluffy. Inoculating a bean plant with the fungus, and re-isolating after infection takes place, brings back these original characters.

<sup>2</sup> It is interesting to note that within this variety Dr. Jones finds strains which show distinct differences in virulence.



3. There are also changes in the microscopic characters, as in the relative number of microconidia and macroconidia produced, and the variation in size and percentage of the variously septate macroconidia. New conidial types also appear. Their characters are again brought back toward the original by inoculating a bean plant with the pathogene and reisolating it.

4. While the six-year-old pathogene was able to infect bean plants, its virulence had been lowered. When the organism was allowed to infect a bean plant and then reisolated, its pathogenicity was not appreciably increased. Two such procedures, however, appeared to restore its former virulence.

5. Such variability in culture both morphological and physiological in the fungus possibly explains the reason for the great number of species and variety in the genus *Fusarium*.

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## OBSERVATIONS ON THE DEVELOPMENT OF THE ROOT SYSTEM OF *ALLIUM CEPA* L.

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### INTRODUCTION

The present paper is concerned with some observations made by the writer on the development of the root system of *Allium cepa* L., in an experiment in which the behavior of onion plants was studied in water and soil cultures. It was observed that the roots formed at the time of germination died at about the time of the formation of the bulb, and that new ones then developed and carried the plants through their complete life cycle.

These observations on the behavior of the onion plants initiated a number of thoughts in the mind of the writer in connection with certain root diseases, particularly those in which the pathogenicity of the causal organism is doubtful. The following studies were made for the purpose of throwing more light on this quite important subject.

### THE DEVELOPMENT OF THE ROOT SYSTEM

Onion plants obtained from two different sources, onion seeds and bulblets or "sets," were employed for these studies. Both the seeds and the bulblets were germinated on sand and then transferred to soil and water cultures. In the case of the water cultures, Shive's best one-atmosphere solution was used for the growth of the onion plants. The soil cultures were prepared with equal volumes of peat and sand mixed thoroughly.

The examinations of the development of the root system in the water cultures were made daily and in the soil cultures weekly. In the case of the water cultures the entire root system was removed from the solution and examined, while in that of the soil cultures only one plant was uprooted from each representative culture. The observations made at different times are recorded in tables 1 and 2. In the tables, those roots formed at the time of germination are designated as "primary roots" and those formed at the time of the development of the bulb as "secondary roots."

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This distinction was necessary in order to avoid confusion as to the behavior of each particular set.

TABLE 1. *Water Cultures*

Stages in the life cycle of onion plants influencing the development, growth, and death of their "primary" and "secondary" roots.

Date, 1923	Stage of Plant Growth	Behavior of the Root System	
		Primary Roots	Secondary Roots
5/12.....	Germination.....	Development of roots	
5/22.....	Planting in water cultures.....	Roots 3-4 in. long	
5/29.....		Roots 5 in. long	
6/6.....		Roots very healthy	
6/13.....	Formation of bulb.....	Roots dying	
6/19.....		More roots dead	
6/26.....		Many roots dead	Roots forming, $\frac{1}{2}$ in. long
7/3.....		About $\frac{3}{4}$ of roots dead	Roots 2 in. long, healthy
7/10.....		Almost all dead	Roots 5 in. long, healthy
7/17.....		Disintegrating	Roots dying
7/23.....		Disintegrating	More roots dead
7/30.....	Plants dying.....	Disintegrating	Many roots dead

TABLE 2. *Soil Cultures*

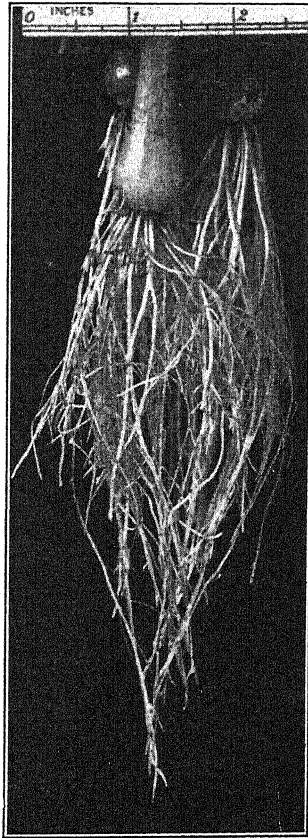
Stages in the life cycle of onion plants influencing the development, growth, and death of their "primary" and "secondary" roots.

Date, 1923	Stage of Plant Growth	Behavior of the Root System	
		Primary Roots	Secondary Roots
5/12.....	Germination.....	Development of roots	
5/22.....	Planting in soil cultures.	Roots 3-4 in. long	
6/6.....		Roots healthy	
6/25.....	Formation of the bulb..	Roots dying	
7/3.....		More roots dead	
7/10.....		Many roots dead	
7/17.....		About $\frac{3}{4}$ dead	Roots forming
7/30.....		Almost all dead	Roots 5 in. long, healthy
8/15.....	Plant dying.....	Disintegrating	Roots dying

### EXPLANATION OF RESULTS

The results shown in tables 1 and 2 indicate that the onion plant, when grown from seed or bulblet, produces at the time of germination a set of roots which grow and function normally until the plant has started forming its bulb. With the gradual development of the bulb some of these roots die; the whole set dying at about the time the bulb is half-formed. The dead roots are succeeded by a new set produced, gradually, at the periphery of the stem. The first set occupies the central part of the stem, the second

the marginal. The second set serves the plant from the stage of the formation of the bulb to the completion of the life cycle. Text figure 1, a photograph taken at the time the onion bulb was about half-formed, shows both sets of roots quite distinctly.



TEXT FIG. 1. Onion plant with its half-formed bulb; "primary" roots dull white, "secondary" roots bright white.

Another very interesting phenomenon is the duration of the different stages in the life cycle of the onion plant in both water and soil cultures. In soil cultures the different stages are longer than in water cultures, and *consequently* the life of the plants is likewise longer in the former cultures than in the latter. The causes which influence this difference in the behavior may lie in the natures of the two different media.

Physiological reasons for the rearrangement observed in the root systems of onion plants can be suggested only after proper consideration of their manner of growth. Growth in onions is centrifugal, *i.e.*, it starts from the center and proceeds outward; it does not differ from the growth of any

ordinary bud. The tissues near the apical region and those which extend over the surface of the stem and support the leaf and storage scales are the youngest and best adapted to regeneration. The tissues directly opposite these, *i.e.*, on the lower surface of the stem, are old, and their regenerative potentiality is relatively low. The primary roots, traced to their origin at the time of their death, appear to emerge from tissues which lie near the lower surface of the stem and are, relatively, old. Their death may be due to a number of causes: (*a*) to the senility of the stem tissues, (*b*) to the senility of the roots proper, and (*c*) to the convolutions which are formed in the tissues of the stem as the result of irregular growth between old and new tissues and which bring about, quite often, disconnection in the water- and food-conducting tissues. In flower-producing plants the "primary roots" do not die rapidly but persist for a longer period.

#### SUMMARY

Onion plants grown in water or in soil cultures produce two sets of roots from the time of their germination to the completion of their life cycle. The first set is produced at the time of germination and functions during the period between germination and formation of the bulb. These roots die gradually during the period of the formation of the bulb. The second set is produced at the time of the formation of the bulb and later, and functions during the period between the formation of the bulb and the death of the plant.

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# OBSERVATIONS ON THE LIFE HISTORY OF *HELICODESMUS*<sup>1</sup>

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(Received for publication June 23, 1924)

This peculiar hyphomycetous fungus, which is in some respects unique among the helicosporous forms of the imperfect fungi hitherto recorded, developed in November, 1921, in a jar culture on twigs of *Salix* which a month before had been found submerged in a small pond between Fresh Pond and Concord Turnpike, Cambridge, and brought to the laboratory. In the following fall, also, the writer, using material from the same locality, again obtained the fungus by making water cultures, both of twigs which had been cast up on shore, and of those partly submerged.

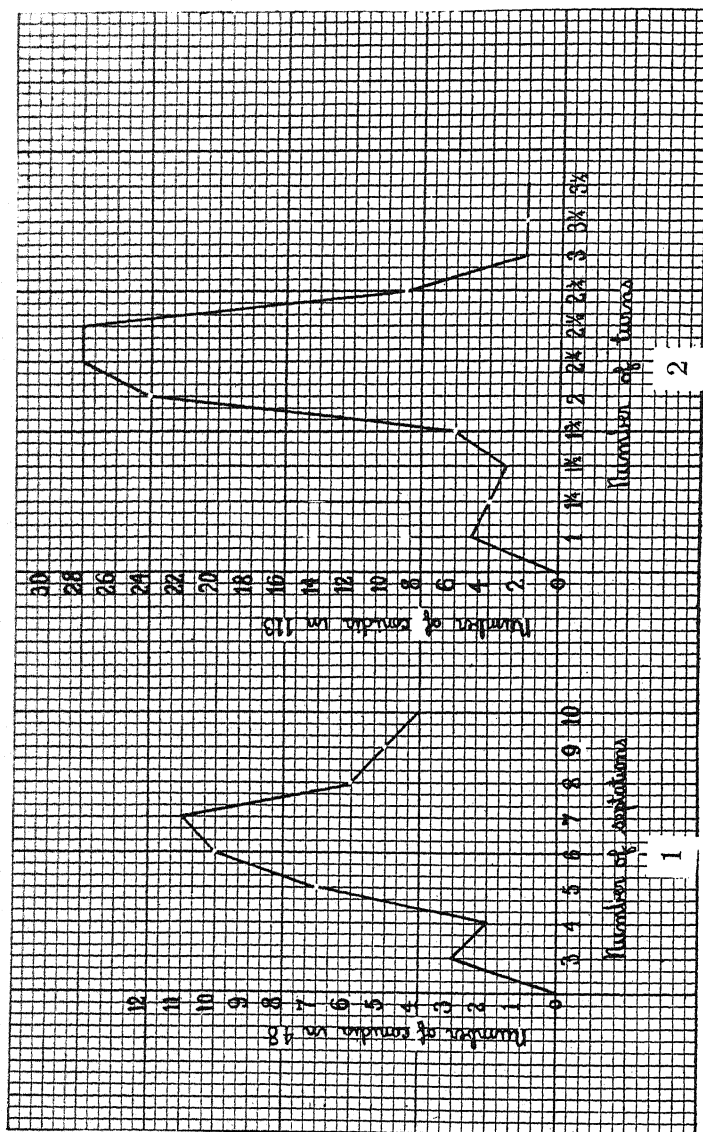
## CHARACTER OF THE FUNGUS

The first indication of the presence of the fungus, both in nature and when thus cultivated, is the appearance of small, white, flocculent tufts of conidia, for the most part borne on that portion of the projecting twigs kept moist by capillarity for about an inch above the surface of the water, but sometimes produced wherever the bark of the twigs is in a saturated condition. Twigs on which the bark has turned brown and is slightly weathered are more favorable for growth than are twigs which are freshly cut. The mycelium runs through the cortical layer and penetrates the xylem by means of the wood rays, from which it is able to grow up or down the twig. When twigs are inoculated artificially, the mycelium is, for the most part, superficial and seldom penetrates farther than the cortical layer. From this mycelium the fertile hyphae arise singly; and whether simple, as is usual, or branched, as is more rare, they bear conidia which proliferate to form the tufts characteristic of this fungus.

The conidia, because of their habit of proliferation, are not borne separately but are tangled into rather complex groups. When young, the developing conidia are crescentic or arcuate and have only two or three cross walls; but as they mature they become helices of two or three complete turns with six, seven, or even as many as ten septa. The conidia, therefore, which one finds entangled when abundantly productive material is mounted, show considerable variation in number of turns and number of septa—comprising from one to more than three coils, usually two to two and one half, and being from thrice to ten times, usually six to seven times, septate.

<sup>1</sup> Contributions from the Cryptogamic Laboratories of Harvard University, XCI.

When, in order to express this quantitatively, the number of septa and their frequency of occurrence in 48 conidia, measured without selection in mounts of optimum material, are plotted in the form of a graph as shown



TEXT FIGS. 1 and 2.

in the accompanying diagram (text fig. 1), it is obvious that conidia with six or seven septations are most frequently encountered. When, in a like manner, the frequency of the number of complete turns or coils in the helices of 113 conidia is plotted graphically (text fig. 2), it is clear that two and a half turns is the preponderating number.



Proliferation of the conidia is a unique but constant character of this species of *Helicodesmus*—a character which has not been observed heretofore among members of the *Helicosporeae*. The process generally does not begin until the spore has become at least one and three-quarters times convolute. A bulge then appears (Pl. XXIV, fig. 16), opposite the point of attachment of the primary spore, or approximately half-way round the first turn, and hence usually from the third, occasionally from the second, cell of the spore. This bulge becomes elongate and finger-like without curving, as if it were a mere hypha of germination, until it reaches a length almost equal to the final diameter of the turn of the primary spore (fig. 17), when it bends and grows in a circle, forming a secondary spore (figs. 18, 19). Shortly after this secondary spore has begun to curve, and while it is elongating, cross walls are formed, at first successively, but later, as the spore matures, without definite order, although never, so far as has been observed, beyond ten in number. Occasionally two secondary spores will grow out at an acute angle from near-by cells of the primary spore, thus giving an appearance of dichotomous proliferation (fig. 11, *a*).

At times, instead of normal spores, peculiar vacuolate, moniliform cells are produced which, in gross appearance, form a soft, waxy mass that is light cream in color. This type of spore is common on rather dry agar slants. If the occurrence of these were confined to that kind of habitat alone, one would be justified in saying that they are abortive results of drying of the substratum, or of a decrease in the humidity of the atmosphere. When, however, one finds the *Monilia*-like form (fig. 13) occurring in a sealed Van Tieghem cell on one side of a colony, and in the quadrant next to that or directly opposite the regular helicoid form is borne typically, then it is difficult to ascribe the cause to drying alone. The moniliform type is obviously an abortive spore, since it proliferates and germinates after the manner of the typical conidium, but is less resistant to drying.

From observations of the fungus during culture experiments, it appears to the writer that proliferation is a purely vegetative phenomenon resulting from the fact that under conditions of high humidity, etc., germination of the conidia starts readily, but, while maximum effort is being put forth for vegetative growth, the process is checked by some external influence, in this case light, which diverts vegetative growth into spore-formation and thus makes conidium-development a repeating process of proliferation.

Germination of the conidia takes place very readily and without a resting period in Van Tieghem cells on plain or nutrient agar or in distilled water. Any cell of the conidium may produce a germination tube which starts out as a bulge, exactly as in the process of proliferation, but soon becomes less refractive than the spores and only slightly more so, for example, than the potato-dextrose-agar substratum. At the end of fourteen hours, at a temperature of 20° to 22° C., germination is well under way—the longest tube in one typical series having attained a length of 48.6  $\mu$

(Pl. XXIII, fig. 2). By the end of twenty-four hours the tube had increased considerably in length, cross walls had been laid down, and the tube had begun to branch (fig. 3). The primary spores of a chain generally germinate first (fig. 2), the others following in the order of formation. Within the spore itself, however, the end cell, when it does germinate, generally does so first, by elongating (fig. 3). In case the terminal cell does not elongate, the order among the cells follows that of the spores, that is, the older germinate first. Very rarely do cells of a conidium send out more than one tube each; while, as far as has been observed, a few of the cells never do germinate. Yet, when a single cell was isolated and grown on nutrient agar, it not only germinated, but in one case was capable of producing two germ tubes (fig. 7).

The mycelium growing out from the germinating conidia into the nutrient substratum forms, in about seven or eight days' development at laboratory temperature, a dense pseudoparenchymatous mat. The development of this mat when followed microscopically is found to occur as follows. About ten or fifteen microns behind the swollen tip of the hypha of germination, a cross wall forms; about ten microns posterior to this, a branch arises (fig. 5). The swollen end of the hypha continues to grow, cross walls continue to be laid down at intervals of 5 to 19 microns, and from each of the cells thus formed, branches continue to arise. These continue their growth, becoming swollen, and behave like the parent hyphae from which they arose (fig. 6) in cutting off cells and forming new septa. This multiplication continues until eventually the pseudoparenchymatous mat is formed, made of elements such as are shown in figure 9, consisting of enlarged, rounded cells that frequently anastomose. Vacuoles now occupy the greater part of the cell, and drops of an oily substance which stain a yellow-orange with Sudan III are numerous. From this mat develop, somewhat later, fine hyphal threads of 2 to 3  $\mu$  in diameter which soon lose their contents and degenerate, and larger hyphae with denser protoplasmic content, 5 to 6  $\mu$  in width. After a very variable length of time, in which fusion takes place between branches of the same hypha or of different hyphae (fig. 10), the conidiophores develop from the mature hyphae in the substratum.

The conidiophores are of no sharply defined type and are not specially differentiated. Most commonly the conidia are borne on short lateral aërial branches which, at intervals, arise singly and for the most part vertically from the repent vegetative mycelium. Usually they are three to eight times septate, simple, and tend to become irregular-arcuate (Pl. XXIV, fig. 11). At times one encounters slight modifications of the usual type, cases in which the conidiophores, instead of being simple, are branched, multiseptate, constricted at the septa, and with basal cells well supplied with oil drops; while the terminal cells are without oil drops and become tortuous, gradually changing into conidia.

## RELATION TO ENVIRONMENT

In culture, this fungus at first showed poor production of conidia, both in Van Tieghem cells and on agar slants. Therefore, in the earlier part of the work, attention was almost exclusively directed towards finding a medium upon which the fungus would grow and produce conidia in abundance. Various solutions were tried. Duggar's solution<sup>2</sup> (2) to which 3 percent of agar was added, was used, not only plain, but also with sufficient asparagin added to the solutions to make concentrations of M/8, M/16, M/32, and M/64. On all of these growth was extremely rapid. With Duggar's solution plus M/8 asparagin, the growth was slightly less than twice as great as on potato-dextrose agar, and nearly twenty times as rapid as on cornmeal agar; but no spores were produced. Potato-decoction agar was then tried with and without the following sugars: lactose, fructose, glucose, and saccharose. There was no apparent difference in reaction to the various sugars, and in all cases abundant mycelium was produced; but again there were no spores. All these cultures, it should be emphasized, were grown in diffuse light as were the cultures on the various modifications of solidified Duggar's solution. With the thought that the natural substratum would be capable of causing, or at least of helping, the production of spores, and also with the idea of testing the specificity of saprophytism of the fungus, twigs of *Salix lucida* Muhl., *Acer rubrum* L., *Betula populifolia* Marsh., *Tilia americana* L., *Sassafras variifolium* (Salisb.) Ktze., and *Helianthus tuberosus* L. were placed in test tubes in the bottom of which were wads of absorbent cotton saturated with water; the whole was autoclaved for 45 minutes at 17 pounds' pressure. This series of material, after inoculation, was divided into two groups, the cotton plugs of one of which were sealed with paraffin; in the other group the plugs were left unsealed. In both groups superficial mycelium was formed abundantly for a short time, and soon appeared to die out. The twigs were inoculated in April, and, with two exceptions caused by drying out of the substratum, the cultures were all producing mycelium abundantly in May. Toward the end of the month the mycelium had begun to die down, and in June had apparently died. The cultures were left thus and were apparently the same when examined again in October. In November they were placed near the window, where it was found in January that the spores were being produced on the *Salix* twigs in the sealed tubes. The other sealed tubes, containing *Acer rubrum*, etc., were examined at the same time and were found to be producing abortive spores. The unsealed tubes, however, had dried out in the meantime without having produced spores. The sealed

<sup>2</sup> Duggar's solution:

M/4 sucrose,  
3M/10 KNO<sub>3</sub>,

M/20 KH<sub>2</sub>PO<sub>4</sub>,  
M/50 MgSO<sub>4</sub> · 7 H<sub>2</sub>O.

The addition of peptone favors mycelial growth, while amino acids favor conidium-production.

tubes were then resealed and left as before. In February these tubes showed sporulation on the various other twigs, thus indicating that, while the natural substratum of *Salix* twigs favors the development of the fungus, it may also grow and reproduce on dead twigs of entirely unrelated species.

This experiment suggested that spore-production was influenced by the season or followed a resting period. To determine the effect of seasonal influences, the effect of humidity was observed in the following experiment. Spores were planted in a series of Van Tieghem cells in the bottom of which were placed different concentrations of sulfuric acid in order to obtain varying degrees of humidity. Spores failed to germinate unless the humidity was above 49 percent; while with the spores that germinated before the cell came into equilibrium, presumably because of the large amount of agar surrounding the spore, growth soon ceased—a fact in agreement with the moisture requirements of the fungus in its native habitat. On the other hand, while the fungus grew wonderfully where there was relatively high humidity, as is the case in the fall, no spores were produced. Therefore, it seems safe to conclude that humidity is a factor in growth, but not in reproduction.

Still working on the seasonal hypothesis, the effect of temperature was noted. Van Tieghem cells were grown in the dark and in diffuse daylight at laboratory temperature, which was maintained during the day at between 20° and 22° C., dropping eight or ten degrees lower at night. At the same time, cells were grown in an incubator, also in the dark, at 29° C. As was to be expected, growth was more rapid at the higher temperature; but there was no evidence of conidia.

Working on temperature led to an effort to discover the methods by which the fungus overwinters. For this purpose, spores on bark in a loosely stoppered bottle, and growing mycelium, both on agar slants and in Van Tieghem cells, were placed outside the window on January 3, and were not taken in for examination until March 28. During this period the records of temperature at the weather bureau in Boston show that as low a temperature as 15° F. (− 9.4° C.) was relatively common, while as low as 2° F. (− 16.6° C.) was reached. There may have been slight differences between the records for Boston and the temperature of the cultures in Cambridge. If so, it is probable that the Cambridge temperatures would be the colder because of the fact that the cultures and that part of the building where they stood were exposed to the sun for only slightly over an hour daily—in the early morning—and because the cultures were placed on an exposed ledge outside the window where radiation would have very little effect. After this exposure, the mycelium on the agar slants in the test tubes was capable of continued growth when tested, even though the agar on which it was growing had lost its jelly-like structure and had become spongy and water-soaked. The spores, on bark in the bottle, germinated with only slightly impaired vigor when brought under

laboratory conditions again; while in one of the Van Tieghem cells spores were produced even during exposure.

Temperature relations of the *Helicosporeae* have not been extensively studied. Rand (8) shows as a result of his work with *Helicosporium nymphaearum* that this species may make a slight growth at 2° C. when germination had occurred before refrigeration; but that there is no growth when transfers are kept at that temperature from the beginning. Furthermore, he finds that production of conidia could not, or at least did not, take place until the temperature rose to 8° or 9° C. At all events, the results of the present experiments show that *Helicodesmus* is resistant to very low temperatures; and, being so constituted, is capable of overwintering as mycelium in infected twigs, or as spores. In nature, also, either as mycelium or as spores, it is probably submerged by the high waters of winter—a situation affording protection from such extremes of temperature.

The effect of light on conidium-production was also studied. Cultures were placed near an east window where they were exposed to direct sunlight during the early morning hours. In these, conidia were produced for the first time in quantity, in less time than was required for their production when the fungus was grown in diffuse light, even though the mycelial growth was slower. Later in the year, cultures were maintained in a west window where they were also exposed to direct sunlight from 2 P.M. until sunset. These cultures, grown on agar slants made from extracts<sup>3</sup> of the green twigs of *Betula populifolia* Marsh., *Salix lucida* Muhl., *Sassafras variifolium* (Salisb.) Ktze., and *Quercus alba* L., and also on the same potato-dextrose agar previously used, all produced spores on the fifth day—a very rapid production for this fungus. Similar cultures on cherry (*Prunus serotina* Ehrb.) agar, however, failed to grow further after germination had taken place.

To verify the dependence of spore-production on light, four tubes were placed in a light-tight box, and four were left outside as close to the box, and under conditions otherwise as nearly the same, as possible. At the end of two weeks the exposed cultures had produced a beautiful, white, zoned veil of spores; while the cultures in the dark had made abundant mycelial growth with but very few spores around the point of inoculation.

Zonation in the growth of the fungus on artificial media was very marked, and was at first thought to be due to the alternation of day and night. Different cultures of the same age had different numbers of rings, however, which made this correlation impossible; and since temperature and humidity varied with day and night, these factors were also excluded. In the experi-

<sup>3</sup> The extracts were made by allowing 250 grams of freshly cut twigs to simmer for an hour in 500 cc. of water. At the end of the hour, the liquid was strained through several layers of cheese-cloth, and the water lost through evaporation was restored. To this extract was added 2 percent of agar which had been previously stirred into a paste with cold water. The extract agar was allowed to boil for a short time, placed in tubes, and then sterilized in the autoclave for 15 minutes at 17 pounds' pressure.

ments confirming light as a causative agent in spore-production, it was noted that there was a zoning in those cultures kept in the dark as well as in those grown in daylight and dark. Moreau (4, 5) describes the same phenomenon.

Microscopical examination showed that these zones on the writer's cultures consisted of concentric rings in which there was much branching of the mycelium, alternating with rings in which there was little. It was further noted that cultures kept in the dark or in diffuse light, conditions favorable to growth, produced broader rings. All this is summed up in Stevens' (10) explanation of the phenomenon, namely, that, because of repeated branching, the fungus becomes crowded and growth is inhibited either by products of metabolism or by exhaustion of the nutriment. The rapidity of formation of the zones is dependent solely on the relation which rapidity of branching bears to rapidity of growth—conclusions substantiated by Brown's (1) recent work.

The Helicosporeae as a group have been investigated but little. New species have been all too briefly described in miscellaneous journals from which they have been abstracted by Saccardo (9). During the process of abstraction, the descriptions were for the most part made so brief that accurate identification of a species is frequently impossible. The European species have been compiled in much the same fashion, but with slightly more ample descriptions, by Rabenhorst (7); and also, with brief mention of more prominent species, by Lindau (3). The American species have been discussed by Morgan (6), with rather full descriptions of several new species and with illustrations. Since Morgan's paper, very little study has been made of this group, especially in the life history of its members. This remained for Rand (8) to accomplish with his very interesting paper describing for the first time *Helicosporium nymphaearum*, a parasite on pond lilies. Its relation to environment, its cultural characters, and parasitism are well worked out; but perhaps the most interesting fact discovered was the occurrence of sclerotia—a phase restricted, so far as the writer is aware, to that species. No spore forms, however, were found in connection with the germinated sclerotia.

The species here under discussion differs markedly from all others hitherto described in the references mentioned, especially in two characters. The first distinguishing character is the production under natural conditions of obvious white tufts of conidia; whereas in other species the spores and sporophores form a diffuse, velvety layer over the substratum. The second point which distinguishes this species is a proliferation of the spores in the manner already described. This method of reproduction is unique among the Helicosporeae and is constant under varying conditions. Thus it seems justifiable to create the new genus *Helicodesmus* to contain the single species which produces its spores in chains.

## DESCRIPTION OF THE FUNGUS

**Helicodesmus** n. gen. Hyaline helicoid spores produced in chains on simple or sparingly branched conidiophores. Vegetative mycelium repent, hyaline, septate, and much branched.

**Helicodesmus** gen. nov. Conidiis in catenulis, hyalinis, in spiras convolutis; hyphis fertilibus hyalinis, simplicibus vel parce ramosis; hyphis sterilibus hyalinis, septatis, repentibus, multo ramosis.

Type species:

**Helicodesmus albus** n. sp. Forms white flocculent tufts on the substratum. Vegetative hyphae hyaline, septate, and much branched, immersed in the cortex of dead, water-soaked *Salix* twigs. Conidiophores arise singly as short, hyaline, mostly simple aërial branches bearing conidia acro-pleurogenously. Conidia in tangled, furcating chains, hyaline;  $1\frac{3}{4}$ - $2\frac{3}{4}$ -times convolute; 6-7-, seldom 3-10-times septate; smooth; slightly constricted at the septa; rounded-tapering at the base; apex blunt and round; conidia  $3.6$ - $6.2\ \mu \times 50$ - $120\ \mu$ ; diameter of the coil  $16.2$ - $25.4\ \mu$ .

**Helicodesmus albus** sp. nov. Albidus, flocculosus, caespitosus; hyphis sterilibus hyalinis, septatis, multo ramosis; hyphis fertilibus brevibus, hyalinis, septatis, plerumque simplicibus, conidiis acro-pleurogenis suffultis; conidiis in furcatis implicatisque catenulis, hyalinis, in  $1\frac{3}{4}$ - $2\frac{3}{4}$  spiras convolutis, 6-7-septatis, rare 3-10-septatis, laevibus, ad septa leviter constrictis, basis rotundato-attenuatis, apice rotundatis; conidiis  $3.6$ - $6.2\ \mu \times 50$ - $120\ \mu$ , helicibus  $16.2$ - $25.4\ \mu$  diam.

In cortice *Salicis lucidae* in aqua jam immersa, Cantabrigia, Massachusetts, America Borealis.

In the two years' culture work, during which the fungus was tried on many different media and under varying conditions, no perfect stages were found. It was not until very late in the second year that the conditions assisting spore-production were discovered—too late to perform the extensive experiments required to cast more light on certain points. It is, therefore, with the realization that many interesting problems are barely touched upon, and some even omitted, that the writer offers this paper in the hope that it may lead to further study on the beautiful and extremely interesting group of fungi imperfecti. In closing, the writer wishes to express his gratitude and indebtedness to Professor William H. Weston, Jr., not only for his guidance throughout the work, but also for placing this remarkable fungus at the writer's disposal for study. Many thanks are also tendered to Dr. Roland Thaxter and to Dr. Carroll W. Dodge for their many helpful suggestions, and to Mr. Eliot C. French for meteorological data and assistance.

## SUMMARY

1. *Helicodesmus albus* n. sp. was found at Fresh Pond, Cambridge, Mass., on the water-soaked zone of partially submerged and weathered *Salix lucida* twigs extending just above the level of the water.

2. The life history consists of two stages: conidial and pseudoparenchymatous. As yet no perfect stage has been found. In addition to normal conidia, abortive conidia are frequently found.

3. The fungus grows well on a wide range of nutrient media, although not on cherry extract solidified with agar, and is little affected by a change in sugars. Growth varies directly with humidity and temperature, and inversely with the amount of light.

4. The fungus is capable of withstanding extremely low temperatures (around 2° F. or - 16° C.), and thus may overwinter by means of perennial mycelium or conidia.

5. Culture experiments indicate clearly that conidium-production is dependent on exposure to sunlight.

6. The fungus is apparently rare, having been found only in the type locality. It is differentiated from other members of the group by the fact that its spores proliferate extensively, producing tangled and furcating chains which form large, white, flocculent tufts scattered over the surface of the saturated substratum.

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### EXPLANATION OF FIGURES

#### PLATE XXIII

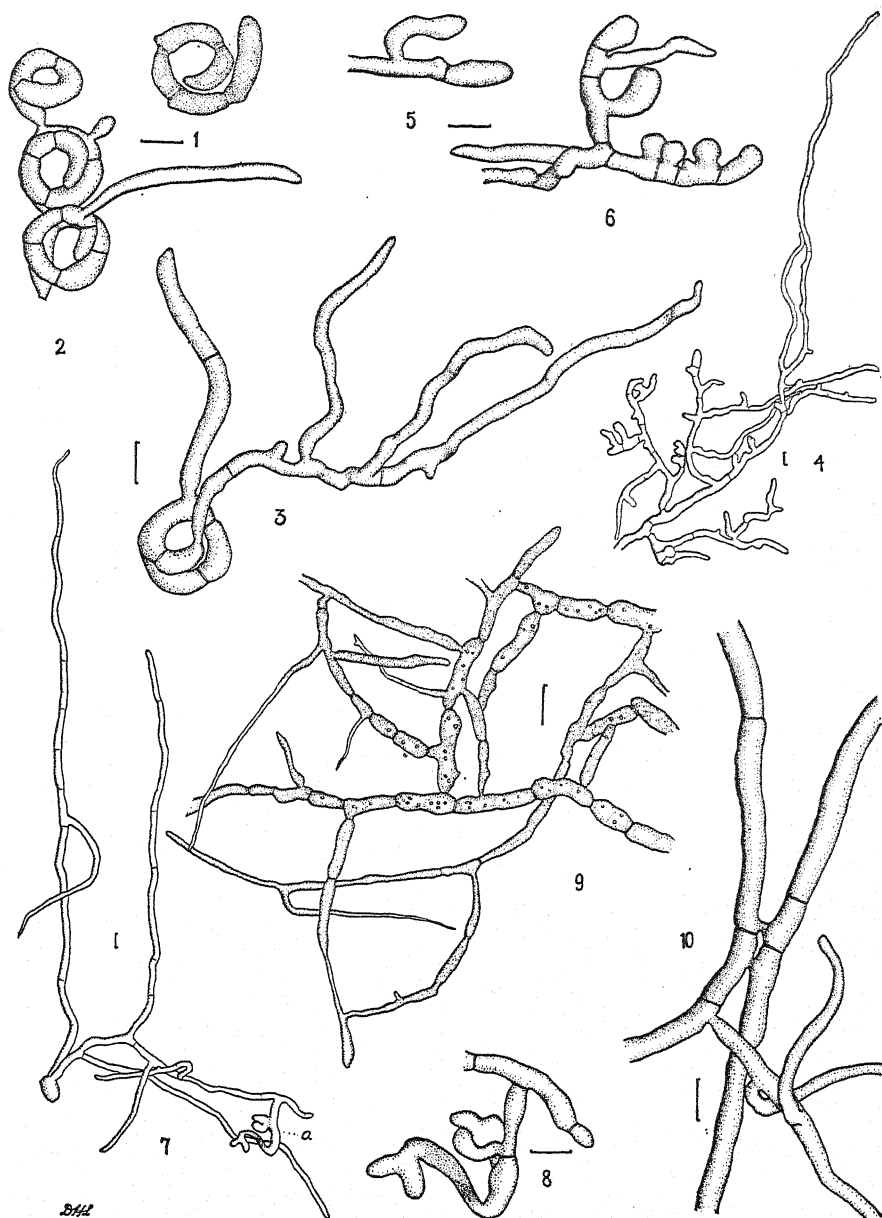
All the drawings on this plate were made from living material except figure 1. Scale = 10  $\mu$  in each case.

FIG. 1. Conidium showing germ tube beginning as a small protruding knob. Note the vacuolate appearance of the contents of the spore. Mounted in glycerin and eosin.  $\times 550$ .

FIG. 2. A chain of spores to show order of germination. The primary spore germinates first, and the others follow in order of their formation. Drawing made 14 hours after inoculation.  $\times 550$ .

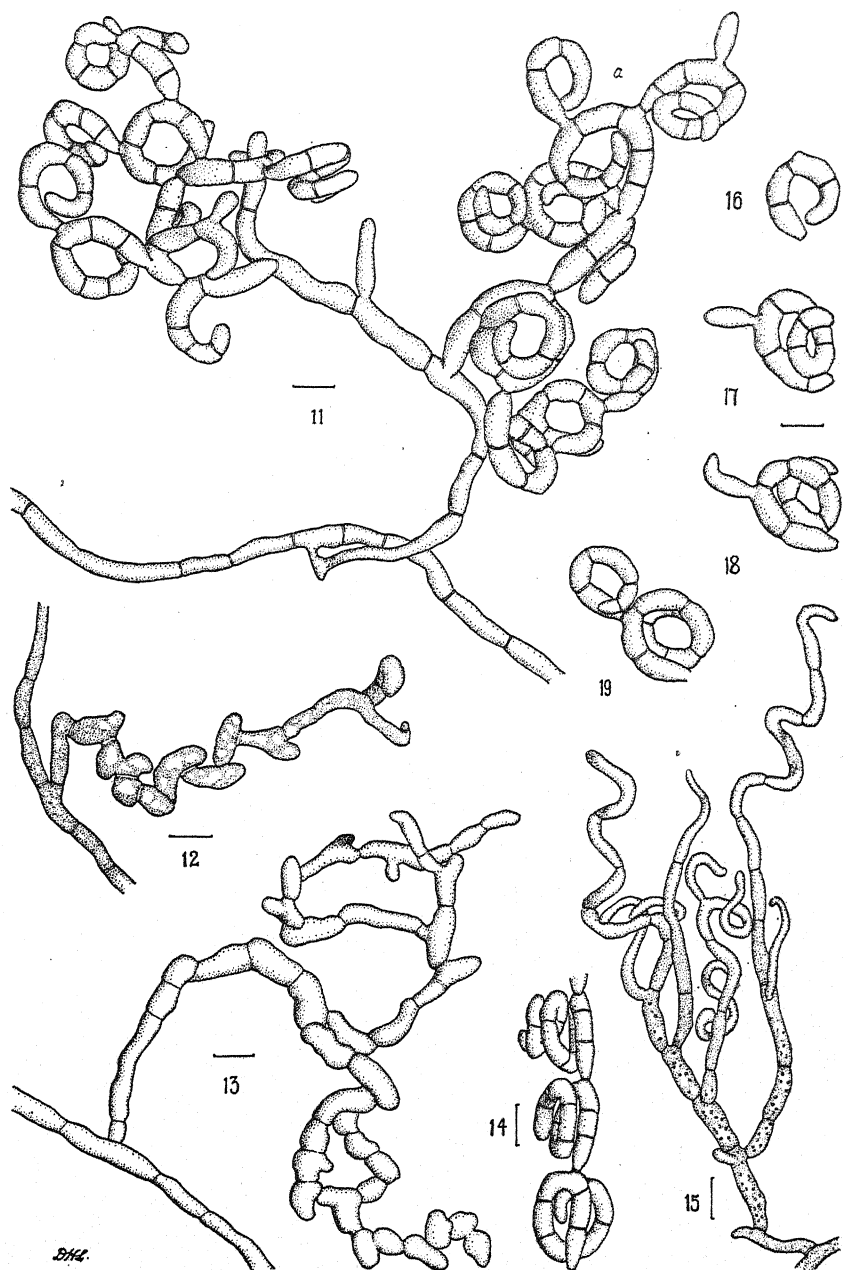
FIG. 3. Drawing to show branching and septation 24 hours after planting the spores.  $\times 550$ .





LINDER: LIFE HISTORY OF *HELICODESMUS*





LINDER: LIFE HISTORY OF *HELICODESMUS*



FIG. 4. Low-power drawing to show the pseudoparenchymatous mat commencing to form. The short, swollen cells may be seen in various stages of formation.  $\times 187$ .

FIGS. 5, 6. Swollen cells more highly enlarged to show stages in the multiplication to form pseudoparenchymatous mat. Figure 5 shows the branch becoming enlarged and septate, and forming knobs after the manner of the swollen tip.  $\times 550$ .

FIG. 7. Low-power drawing to show germination of a single cell of a conidium by two germ tubes. The pseudoparenchymatous mat may be seen commencing to form at *a*.  $\times 187$ .

FIG. 8. Swollen cells of figure 7 *a* more highly magnified to show the difference from the swollen cells in figures 5 and 6.  $\times 550$ .

FIG. 9. Elements from a pseudoparenchymatous mat, showing the enlarged vacuolate cells with oil drops. At the left of the figure may be seen fine hyphae that taper and soon lose their contents.  $\times 550$ .

FIG. 10. Fusion between two different hyphae following the pseudoparenchymatous stage.  $\times 550$ .

#### PLATE XXIV

FIG. 11. The simple arcuate type of conidiophore is shown arising from the repent sterile mycelium and bearing two groups of spores pleurogenously, with a third group starting between these as a conidium that has not yet curved or become septate. The main axis of the conidiophore is soon to be terminated by another spore or group of spores. The secondary spores originate opposite the place of attachment of the primary spores. When a second group of secondary spores arises from one primary spore, it comes off opposite the first group of secondary spores, except at *a* where incipient dichotomous branching of the spore chain is illustrated.  $\times 550$ .

FIG. 12. A simple type of conidiophore, bearing a chain of moniliform aborted conidia at the tip of which a typical helicoid conidium may be seen in an early stage of its development.  $\times 550$ .

FIG. 13. Moniliform or abortive spores borne on the typical simple conidiophore. Drawn from the same culture as figure 12.  $\times 550$ .

FIG. 14. Side view of a chain of conidia to show the 2 to 3 coils of the helices.  $\times 550$ .

FIG. 15. The less common branched type of conidiophore with spores commencing to form at the tips of the sinuous branches. Note the vacuolate cells, with oil drops, in the main axis at the bases of the branches of the conidiophore, also the definite constrictions at the septa of these cells. Progressing towards the tips, the branches become more refractive as the protoplasm becomes less dense.  $\times 550$ .

FIGS. 16-19. In figure 16 is shown a bulge that is to be a secondary spore, at the side opposite the base of the primary spore. This bulge grows out straight until it reaches approximately the same diameter as the primary spore, as is shown in figure 17. The elongate knob now curves over (figure 18), and cross walls are laid down successively as the secondary spore elongates. After the four-celled stage (the five-celled stage is illustrated in figure 19), cross walls may be laid down within the cells already formed; or the cells may be cut off from the elongating terminal cell as was the case at the beginning of septation of the spore now formed.  $\times 550$ .

# STUDIES ON THE COMPARATIVE CYTOLOGY OF THE ANNUAL AND BIENNIAL VARIETIES OF *MELILOTUS ALBA*

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## INTRODUCTION

Although the biennial variety of *Melilotus alba* has been known in the United States for a long time, the annual variety has been found only within recent years (28). The annual may be distinguished from the biennial variety in that the food and energy in the annual are used to form a main central vertical stem with smaller lateral branches, whereas in the biennial, although they form a central vertical shoot, greater emphasis is placed upon the production of numerous lateral branches. From buds on the crown of the biennial plant, lateral branches arise during the second season and produce numerous flowers. In view of the fact that the two varieties are functionally different, the present investigation was undertaken to ascertain whether any cytological differences exist between them.

## MATERIALS AND METHODS

Material was collected from plants grown both in the greenhouse and in the field. The entire study was made from young flowering racemes of both the annual and the biennial varieties, taken when the racemes were from 0.5 to 1.5 cm. long. The material was killed and fixed in chrom-acetic acid of medium strength, or in medium chrom-acetic acid to which were added ten drops of two-percent osmic acid per 50 cc. of solution. Sections were cut 3 to 10  $\mu$  thick, stained either with Heidenhain's iron-alum haematoxylin or with Flemming's triple stain. Much of the material was studied in living as well as in fixed condition. Chromosome counts were made both in sections of fixed material and in the living condition by the use of Belling's (3) iron-aceto-carmine method.

## THE ANNUAL VARIETY

The development of the anther is, in general, similar to the usual method of anther-development, except that the spore mother cells of *Melilotus* are formed by the continued division of but a single longitudinal row of sporogenous cells in each anther lobe rather than by the division of the whole transverse row.

### Resting Stage and Synizesis

In the sporogenous cells just previous to the formation of the mother cells the chromatin appears in small clumps, most of which are in contact with the nuclear membrane. Two nucleoli are frequently seen in each nucleus at this stage.

The pollen mother cells are polyhedral in shape and their cytoplasm is more dense than that of the surrounding cells. The nuclei in the resting stage are 5 to 7  $\mu$  in diameter. The chromatin is at first rather clumped just within the nuclear membrane, but it soon assumes the form of a loose reticulum (Pl. XXV, fig. 1), in the midst of which is a single nucleolus, which exhibits a differentiation of structure into a central region which is somewhat transparent and a peripheral region which stains deeply (fig. 1). During the resting stage, as well as in succeeding stages, the nucleolus shows little buds (figs. 1-4) being constricted off at its surface, suggesting that nucleolar material is given off into the nuclear sap and that it may be used in the further development of the nucleus.

During the development of the leptonema stage the nuclear reticulum assumes a more distinctly thread-like appearance (fig. 2). Because of the small size of the nucleus, the writer was unable to follow further the behavior of these threads and can make no statement as to whether or not they pair at this stage. The first evidences of synizesis are the appearance of a clear space at one side of the nucleus and the thickening of the threads of the chromatin network. The threads now contract, forming a very tight knot at one side of the nucleus, in which the nucleolus is at first entirely imbedded but from which it soon emerges (fig. 3). The synizetic ball evidently remains intact for a relatively long time, as shown by the large number of nuclei found in this stage. Later it loosens and appears as a system of anastomosing threads (fig. 4). The threads become rather uniformly distributed throughout the nucleus and are certainly now much thicker in the pachynema (fig. 5). No second contraction stage was observed.

### Diakinesis

The thread now breaks up into a number of irregular pieces, the chromosomes (fig. 6), which soon become more regular in outline and either bend in such a way as to have one end of the chromosome fold over the other end, or assume the shape of a U. In either case a break occurs at the bend of the chromosome, thus forming the two components of the bivalent chromosome. These components at first become so arranged as to form a figure X or V or O, but eventually come to be in contact throughout their entire length (fig. 7). In diakinesis the writer has been able to establish beyond doubt, both by counts in sections of fixed material and by the use of Belling's (3) iron-aceto-carmin method for living material, that the number of bivalent chromosomes is eight (fig. 7). This diakinetic figure clearly shows

that the chromosomes are double. One of these chromosomes is U-shaped and is in the stage just previous to breaking at the point of greatest curvature to form the two components of a bivalent chromosome.

### The Heterotypic Division

As the heterotypic division is initiated, the nuclear membrane and nucleolus disappear. The chromosomes at first become arranged parallel with the long axis of the heterotypic spindle on which, however, they soon assume various positions (fig. 8). Figure 9 represents a section cut at right angles to the long axis of the heterotypic spindle through the equatorial region, and shows a cross section of the eight bivalent chromosomes. Many of the preparations in this stage show the number of bivalent chromosomes to be eight. The members of each bivalent chromosome now separate along their line of contact and go to the opposite poles. In the anaphase of the heterotypic division the chromosomes are closely grouped together (fig. 10), presenting a rather flat chromatin mass in which it is often impossible to distinguish the individual chromosomes. They soon become separated, however, and the group assumes a more spherical form, at which time one can without difficulty see that there are eight chromosomes at each pole. Each nuclear mass becomes enclosed by a membrane, and it is still possible to see that there are eight chromosomes in the nucleus (fig. 11, Pl. XXVI). As the daughter nuclei enlarge slightly, the chromosomes become more or less completely fused together (fig. 12), but soon break up into numerous small clumps. A nucleolus is formed in each of the daughter nuclei, which now show a more or less definite chromatin network (fig. 13). Thus, contrary to the usual situation at the end of the heterotypic division, the nuclei are completely reconstructed. By this time the spindle fibers of the heterotypic spindle have entirely disappeared and there is no evidence of even a trace of a cell plate or wall or cleavage furrow across the spindle at any time.

### The Homoeotypic Division

The daughter nuclei remain in the resting stage (fig. 13) for only a short time. The next succeeding stage observed is represented by figure 14, in which the univalent chromosomes of the daughter nucleus are seen to be separated at their ends. A deeply staining nucleolus is also seen. Just what the procedure between the resting stage (fig. 13) and the reappearance of the univalent chromosomes is, the writer has been unable to determine as the preparations lack the intervening stages. The nuclear membrane and nucleolus of each daughter nucleus disappear and the univalent chromosomes become arranged on the homoeotypic spindles. The spindles are usually at right angles to each other (fig. 15), although they may occasionally be parallel. Figure 15 represents a section cut through the equatorial region of one of the spindles, through the chromosomes, and clearly shows



the number of chromosomes to be eight. Although no evidence of a split in the chromosomes is seen in this section, they do split very soon after this stage and the components of each univalent chromosome go to the opposite poles. In the anaphase of the homoeotypic division the chromosomes are at first closely massed together, just as in the heterotypic. They soon become separated and can be counted before the nuclear membrane is formed. A membrane is soon formed around each of the four nuclei, and here again the number of chromosomes is seen to be eight (fig. 16). A nucleolus is formed in each nucleus at this stage. Soon the chromosomes break up into small fragments which are distributed throughout each nucleus. It is interesting to note that in this stage-four, and in the similar stage of the heterotypic division two, deeply staining bodies not unlike centrosomes are seen in the cytoplasm (figs. 11, 16). The writer has been unable to ascertain either the origin, the function, or the final disposition of these bodies. The nuclei at the end of the homoeotypic division are tetrahedrally arranged and are connected by six spindles.

### The Wall of the Pollen Mother Cell

The pollen mother cells are at first polyhedral in shape and each is surrounded by a thin membrane. During synizesis a clear homogeneous substance is secreted by the protoplast which is at first seen only at the corners of the mother cell just as synizesis is initiated (fig. 3, Pl. XXV). As the development of the nucleus proceeds, the protoplast becomes entirely surrounded by this homogeneous substance (fig. 4), until in the pachynema it becomes quite massive (fig. 5). During the secretion of this wall the protoplast assumes a spherical form. Mangin (27) first showed in *Carex* and *Juncus* that this wall is composed of callose, and further evidence of the nature of this wall is given by Beer (1) who demonstrated in *Oenothera biennis* and *Aucuba japonica* the presence of a similar pollen mother cell wall composed of pure callose. By the use of most of the microchemical tests used by Mangin and by Beer, namely, ruthenium red, lachmoid, anilin blue, corallin soda, congo red, caustic potash, and sodium hydroxid, the writer has found this massive wall in *Melilotus* to be composed of practically pure callose.

### The Furrowing Process

No further change occurs in this callose wall until the end of the homoeotypic division. Figure 16 shows the condition of the protoplast as the homoeotypic division nears completion. The spindle fibers are quite conspicuous in this stage, but they soon begin to disappear (fig. 17, Pl. XXVI). Infoldings now occur at the periphery of the protoplast, equidistant from the nuclei, and at right angles to the former spindles. The subsequent behavior of the protoplast in the formation of the microspores is

very unlike that ordinarily observed in microspore-formation, and a detailed description of the process will therefore be given.

It had long been thought that the quadripartition or simultaneous division of pollen mother cells in dicotyledons occurred by means of cell plates. Farr (8) has shown that quadripartition in certain dicotyledons, as well as in one monocotyledon, occurs by a process of furrowing after the homoeotypic mitosis is complete, and he believes that in no case is quadripartition of the pollen mother cells of dicotyledons effected by means of cell plates. In *Nicotiana* at the end of the homoeotypic division a furrow is formed along the equator of each spindle. As the furrows proceed from the periphery toward the center of the mother cell there are four equidistant invaginations of the mother cell wall which eventually meet in the center of the tetranucleate cell, dividing it into four uninucleated protoplasmic masses. Thus the partition walls between the four microspores are continuations of the wall of the pollen mother cell.

In *Melilotus* at the end of the homoeotypic mitosis the spindle fibers become completely resorbed, and the protoplast is seen to contain numerous small refractive vacuoles which are uniformly distributed and which are apparently filled with cell sap. Many of these vacuoles persist in the cytoplasm even after the four microspores are independent of each other (figs. 18, 20), but disappear as the pollen grains begin to develop. Soon less dense areas are recognizable in the cytoplasm, extending midway between the nuclei and reaching from the periphery to the center of the protoplast. Rows of vacuoles, larger than those previously mentioned, are now seen to extend across these hyaline areas (fig. 18). While this takes place there is evidently an increase in the density of the cytoplasm in the vicinity of the nucleus, which is due to the movement of cytoplasmic material from the regions of cytoplasm equidistant from the nuclei. The vacuoles as seen in figure 18 soon fuse, forming larger vacuoles which are variable in shape, thus leaving only a few strands of cytoplasm connecting the four protoplasmic masses (fig. 19). Finally the few connecting strands become severed by incoming surface furrows, and the microspores thus become separate protoplasmic masses with wide furrows between them. The new surfaces at the edges of the protoplasts adjoining the furrow are rough and irregular, caused by the rupture of the strands of cytoplasm which connected the microspore masses. The furrows soon become narrower and more regular in outline, in consequence of the turgor of the protoplasts, as the newly formed plasma membranes adjoining the furrow approach each other.

This method by which the cleavage of the cytoplasm occurs in *Melilotus* is not unlike that observed in the *Phycomycetes* and *Myxomycetes* by various workers. In *Pilobolus* Harper (15) observed a number of small vacuoles arranged in a dome-shaped layer parallel to the periphery of the sporangium. These vacuoles fuse edge to edge, forming a furrow which,

aided by a cleft starting at the periphery, cuts out the columella. Only a few strands of protoplasm connect the spore-plasma with that of the columella as the furrowing process which delimits the columella nears completion. Surface furrows now progress inward, meeting the vacuoles, thus cutting the plasma into irregular multinucleated masses. In Sporodinia the two membranes bordering the cleft which cuts out the columella approach each other and a wall is laid down between them. Swingle (40), working on *Rhizopus* and *Phycomyces*, and Schwarze (35), working on *Sporodinia grandis* and *Mucor mucedo*, observed the same general situation in spore- and columella-formation in the sporangia.

The mechanics of cleavage and furrowing has received considerable attention by various workers. Bütschli (4) interpreted furrowing and cell division as the result of a higher surface tension at the equator of the cell, caused by the flow of protoplasmic currents toward the centrosomes, and the work of McClendon (24-26) adds credence to this theory; whereas, on the contrary, Robertson (29-31) considers furrowing as due to a decrease in surface tension at the equator, caused by the diffusion of materials from the nuclei toward that region. That Bütschli's observations were probably correct is shown by the work of Spek (36, 37), who, by using droplets of oil and mercury in water, was able to imitate furrowing by lowering the surface tension at the two poles of the droplet, thereby increasing the surface tension at the equator. Moreover, he observed streamings in the droplets and in dividing eggs. This interpretation is further corroborated by Chambers (5), who found that two semi-solid masses are formed at the poles, and that the elongation of the egg is caused by the growth of these masses. Finally a cleavage furrow develops in the more fluid portion of the egg substance midway between the daughter nuclei. The highest surface tension is, of course, in this more fluid region. In this same connection, Kite (21), from a preliminary study of cell division, concludes that it is very largely the result of "concomitant shrinking and swelling or change in water-holding power of different portions of the cytoplasm." Recently, however, Gray (13) has shown that it is unnecessary to assume the occurrence of regions of differential surface tension on the cell surface. Using fertilized animal eggs, as well as two drops of oil in acid and normal sea water, he found that the shape of the dividing cell is the result of an equilibrium between a force inside the cell and surface tension. He attributes cell division to the movement of two asters away from each other, and maintains that the cleavage furrow is due to an equilibrium between the effect of this movement on the protoplasm and the surface tension on the surface of the cell. Farr (8) thinks that the nuclei, after the second division of the mother cell, behave as though bearing electrical charges of like sign, thus repelling each other, whereas the plasma membranes bear charges of opposite sign and are attracted toward each other. While this theory seems plausible, it will doubtless lack general acceptance until more experimental evidence is at hand to substantiate it.

The matter has been approached from a different angle by other workers who find that the furrowing process is, in many cases, to be attributed to the fusion of vacuoles. Harper (15) is of the opinion that cleavage might be connected with the loss of water and indicates the similarity of surface furrowing to the cracking of the surface of a drying mass of a colloidal substance. He considers this explanation by itself as inadequate, for the multinucleated mass is segmented with reference to the distribution of the nuclei, and he suggests that the regular segmentation of uninucleated masses might be attributed to less loss of water in the vicinity of the nuclei than elsewhere. In a later paper, Harper (17) connects this with the effects of alkali and acid on the imbibition of water by colloids, and suggests that a localized concentration of acid in the spore-plasm, involving a differential water-holding power, would determine the orientation of the cleavage furrows, since the cleavage planes would follow those zones containing the least water, thus delimiting the acid-containing areas. If the chemical nature of the nucleus should make it a center of water concentration, uninucleated spores would, therefore, be produced. Swingle (40) has explained the cleavage process in *Rhizopus* and *Phycomyces* on the basis of localized contractions of the cytoplasm and does not consider the nuclei as directly influencing contraction. The explanation is very simple, but there is no suggestion as to the origin or cause of these local contractions.

The evidence in *Melilotus* indicates that the planes of cleavage are pre-determined by hyaline areas located midway between the nuclei, and extending from the periphery in a manner described by Harper for *Pilobolus* (15) and for *Fuligo* (16), in which these hyaline areas extend between the nuclei after the early stages of cleavage have been initiated. W. K. Farr (12) found similar hyaline areas extending across the equators of the spindles of the pollen mother cells of *Cobaea*, which areas were accentuated by denser areas around the nuclei. In *Melilotus* these hyaline areas are apparently due to the movement of the granular material from these regions of the cytoplasm to the vicinity of the nuclei. This is accompanied by the extrusion of liquid into vacuoles which fuse, forming larger vacuoles, thus leaving the cytoplasm of the four masses of protoplasm, which are to become microspores, connected by only a few strands of cytoplasm. These strands are soon severed by surface furrows which originate at the periphery. The nuclei play an important part in determining the planes of cleavage, and, since the movement of granules toward the nuclei would be initiated at regions equidistant from the nuclei, it is evident that the cleavage planes would be formed at these regions. It is clear that the cleavage furrows are formed almost entirely by the fusion of vacuoles and that the furrows which originate on the surface do not progress centripetally until the vacuolation is well advanced, and that the furrows proceed but a little distance before cutting into the large vacuoles. This movement of the furrows through the cytoplasm until they meet the vacuoles is doubtless due to higher surface tension in the regions where the vacuoles are seen.

### Formation of the Special Wall

Just previous to the appearance of the hyaline areas in the cytoplasm, a denser, more refractive layer of callose is secreted between the border of the protoplast of the pollen mother cell and the callose wall which has already been described (figs. 17-19). This is the beginning of the *special wall* described by Strasburger (39). As the cleavage furrows are formed by the fusion of vacuoles, the special wall assumes a wedge-shaped appearance (in section) at the periphery of the protoplast at the outer border of the equatorial zone (fig. 19). As shallow furrows are formed by the invagination of the plasma membrane, due to the higher surface tension of the protoplast in these regions, the wedge-shaped regions of the developing special wall follow the invaginating plasma membrane, which advances but a short distance before it cuts into the large vacuoles. The inward movement of the surface furrows and of the special wall is delayed until the furrows formed by the vacuoles are almost completed (fig. 19). After the severing of the strands of cytoplasm, and the narrowing of the furrow, the protoplasts of the young microspores secrete between them a homogeneous substance which is shown by its microchemical reactions to be callose. The blunt wedges of the special wall, which up to this time have protruded into the furrows only a short distance, grow inward from the periphery by the deposition on their inner surfaces of the callose which is secreted by the protoplasts (fig. 20). These partition walls advance centripetally until they meet in the center, and even when they come in contact at the center they are still very narrow and somewhat irregular. By the continued secretion of callose this wall becomes uniformly thickened around the four microspores (fig. 21, Pl. XXVII), finally attaining its maximum thickness as shown in figure 22. This is the mature special wall which surrounds each microspore. In living material, the tetrad of spores can at this stage easily be teased out and studied in the living condition. When mounted in water the original mother-cell wall can easily be distinguished from the special wall. Although both are homogeneous, the special wall is more refractive and more compact. By firm pressure on the cover glass the microspores with the special wall enclosing them can be freed from the mother-cell wall as the former is much more resistant to pressure (fig. 23). By applying a few drops of resorcin blue (lachmoid) solution to living groups of tetrads of microspores, the two walls are easily distinguishable. Both walls are stained a brilliant blue, but the special wall becomes more deeply stained and is more refractive than the mother wall. When fixed material is sectioned and stained with safranin, gentian violet, and orange G, the mother wall takes a pale orange stain, whereas the special wall is much more deeply orange-stained and has the appearance of being more compact. Beer (1) reports that in *Oenothera* septa are developed between the cells of the tetrad, forming an extension of the mother-cell wall. In a later paper

(2) he describes and figures this special wall around the cells of the tetrads of *Ipomoea*, and finds that it is composed of callose and pectose.

The callose mother-cell wall of *Melilotus* described above persists for a short time after the special wall is complete. As the walls of the pollen grains form, however, the mother-cell wall gradually disappears, breaking down into a substance whose chemical nature has not been determined. Eventually the special wall also breaks down, liberating the four microspores. As the callose walls disappear there is a noticeable thickening of the exine of the pollen grains, which rapidly enlarge and in doing so change from a spherical to an elliptical form (fig. 24). The thickening and enlargement continue until the pollen grains are mature. The mature pollen grain has three longitudinal grooves on its surface which are best seen in cross section (fig. 26). On each groove midway between the ends of the pollen grain is a pore (fig. 25), which is formed as the exine thickens by the failure of the deposition of any exine material at this point. The exine stains a deep red when safranin and gentian violet are used, whereas the intine stains violet. In mature pollen grains the nucleus and the cell have divided, and the generative cell is clearly seen (fig. 25).

The tapetal cells are quite large, usually larger than the pollen mother cells, are uninucleated, and have dense cytoplasm. There is no evident change in the structure or contents of these cells until the pollen grains are rather well developed and the exine is forming. At this point, the deeply staining nucleus gradually loses its contents, and soon appears to be no denser than the cytoplasm. It is only when the pollen grains are nearly mature that the tapetum is disintegrated and is represented only by occasional fragments of the protoplast seen among the pollen grains.

#### THE BIENNIAL VARIETY

The foregoing description of microsporogenesis is of the annual variety, but it serves equally well as a description of microsporogenesis in the biennial, for in no case has any difference been found between the two from a cytological standpoint. There is uniformity in number and size of the chromosomes as well as close conformity in all other cytological behavior. That the two varieties are distinct from a morphological and physiological standpoint with respect to their habit of growth, there is no doubt. Attempts were made to induce the biennial variety to flower during the first season by bringing plants into the greenhouse near the end of the growing season. The plants grew very little, and, although kept in the greenhouse for more than a year, did not flower. Other plants were grown in the greenhouse and then taken out into the field in the spring, but in no case did they flower during the course of the entire summer. However, when plants were allowed to remain outdoors until they had been exposed to freezing weather and were then taken into the greenhouse, they immediately began to grow, and formed new shoots which flowered in two and one half months.

If the biennial is left in the field during the winter, it produces new shoots in the spring, and these shoots flower abundantly. Although the annual, if kept under favorable conditions, will continue growth and reproduction for a longer period than it normally does, it is killed by allowing it to remain out of doors over winter. Thus *Melilotus alba* has two functionally distinct types which are alike cytologically in microsporogenesis. Although morphological and physiological differences in plants are often accompanied by chromosome differences, cases also occur in which structural and functional differences appear without corresponding chromosome differences. The work of Täckholm (41) on the genus *Rosa*, of Rosenberg (33) on the genus *Crepis*, and of Sakamura (34) on the genus *Triticum*, illustrate species and varieties within a genus having differences in chromosome numbers as well as in size. Recently Collins and Mann (6) found that a cross between *Crepis setosa*, which has four pairs of chromosomes, and *C. biennis*, which has twenty pairs, produced  $F_1$  individuals which show much less irregularity in microsporogenesis than do the  $F_1$  hybrids of a cross between *C. setosa* and *C. capillaris*, the latter of which has three pairs. They conclude that normality of reduction does not depend upon similarity in chromosome number, but rather upon likeness in internal composition of chromosomes. A similar conclusion was reached by Gregory (14) who, as the result of a study of two giant races of *Primula sinensis*, one of which had twelve pairs of chromosomes, and the other twenty-four pairs, is of the opinion that the results obtained throw no light on the relationship between the characters and the chromosomes. Moreover, Jorgensen (19) recently observed that in *Callitriche stagnalis* some plants have a haploid number of five whereas other plants have ten as a haploid number. Although he considers this species as a composite of more than one species on a cytological basis, it is clear that even so large a difference in chromosome number may not always be associated with corresponding conspicuous morphological differences.

From the consideration of morphological behavior as related to chromosome behavior, it is evident that morphological changes may occur without visible changes in chromosomes. Likewise, there may be variability in chromosomes without corresponding conspicuous morphological changes. The two varieties of *Melilotus alba* exemplify functional differences with no visible corresponding differences in chromosomes or other cytological characteristics in microsporogenesis, and it seems that any explanation of the functional difference must go beyond the chromosome as an entity. There is evidently a difference in factors between the two plants, and these factors are in the writer's opinion beyond the realm of cytological investigation at the present time.

#### ABNORMAL POLLEN

In material which was collected from a field of plants of the annual variety, numerous pollen grains were found the volume of which was from

five to six times that of the normal pollen in the same anther. The abnormal grains have less dense, vacuolate cytoplasm, and pores are absent on the exine (fig. 31). The pollen mother cells which give rise to the abnormal grains are easily identified. At an early stage they stain much less densely than do normal mother cells (fig. 27). This is particularly noticeable in the nucleus, which, although of normal size, seems to be greatly lacking in chromatin, for the reticulum stains a very dull gray whereas normal nuclei in the same section are stained deeply. The nucleolus, however, is deeply stained. When diakinesis is reached the chromosomes are stained very feebly, and frequently all that is seen of the chromosome is a membrane-like edge with a few feebly stained granules on the interior. In this as well as in succeeding stages the protoplast is normally spherical in shape, but here the protoplast has failed to round up and has usually the same shape as that of the mother cell when in the resting stage—that is, polyhedral (fig. 28). The writer's material shows no mitotic figures of either the heterotypic or the homoeotypic division. The next stage observed was after the homoeotypic division had taken place and was represented by numerous mother cells, each with four nuclei (fig. 28). The appearance of the mother cell at this stage is very different from that of a normal mother cell, for the protoplast is still irregular in shape and the nuclei are variously arranged. Rogers (32), studying the biennial variety, reports that one of the four nuclei enlarges and the other three degenerate. The writer's material does not show this condition; some of the oldest pollen grains in the preparations show four large, well organized nuclei (fig. 31), and in no case have any degenerating nuclei been found. Pollen mother cells have been found, however, in which several of the nuclei had fused (fig. 33). Occasionally giant pollen grains are found which show the presence of a generative cell (fig. 32). In anthers containing both normal and abnormal pollen, numerous normal grains have a generative cell, whereas in all other material which contained only normal grains the appearance of a generative cell was extremely rare.

Thus, each pollen mother cell usually forms only one pollen grain which is very large. Occasionally, however, cross walls are seen at various stages of completion, varying from no cross walls to cases in which complete cross walls are formed. Some mother cells were seen in which only one cross wall was formed (fig. 30). In others, no wall was formed but the elongated pollen grain became somewhat constricted in the middle. In other cases, both cross walls were formed, but the four microspores thus formed remained joined together (fig. 29). Whether or not these large pollen grains are capable of germination has not been determined.

Cases in which less than the regular number of pollen grains is formed from a mother cell are few. Wille (43) reports the occasional formation of two from a mother cell in eight different species, and of three microspores in three different species. Instances in which only one microspore results



from a mother cell are very rare. Elfving (7), Wille (43), and Strasburger (38) found in several species of the Cyperaceae that only one functioning microspore is formed from a mother cell, as the other three members of the tetrad disintegrate. Juel (20) observed in *Carex acuta* that in meiosis each division is followed by the formation of a cell plate. These plates are resorbed and the four nuclei lie free within the mother-cell wall, which becomes the microspore wall. Three of the four nuclei then degenerate.

The formation of abnormal pollen is often associated with hybridity, although hybridity can not be considered as the sole cause of pollen abnormality or sterility. In *Melilotus* the writer is inclined to think that the giant pollen grains are due to hybridity between the annual and biennial varieties, for plants have been found in fields of the annual variety which had the appearance of being natural hybrids, since they possessed both annual and biennial characters. In some of the flowers of these plants giant pollen grains have been found, and this occurrence of giant pollen grains in material which seems to be hybrid between the two varieties suggests the explanation of the cause of the giant pollen grains.

#### DISCUSSION

That the two varieties of *Melilotus alba* are distinct is evidenced by their different functional activity which results in their different habit of growth. That the two varieties are distinct is shown by the fact that it is impossible to force the biennial to flower by providing conditions suitable for its continued growth at the end of the growing season, whereas the annual variety continues normal growth and reproduction if suitable conditions are provided as the unfavorable season approaches.

From an examination of published lists of chromosomes in plants and animals it is evident that the number of chromosomes shown by the species of a genus is often variable. The chromosomes are often in multiples, but occasionally species differ by only one or two pairs of chromosomes. On the other hand, these chromosome lists show that there are species of a genus which, although morphologically distinct, have an identical chromosome number. It is evident, therefore, that there are two categories of behavior with reference to chromosomes and morphology. In the first, morphological difference is associated with chromosome difference. In the second, morphological difference in species or varieties is unaccompanied by visible chromosome variation. Since the chromosomes are regarded as the structures in which plant or animal character-determining factors reside, and since no chromosome differences are evident in many cases where morphological differences exist, it is logical to conclude that these morphological differences are brought about and are accompanied by changes within the chromosome itself. The two varieties of *Melilotus alba* would differ, therefore, not only in functional characters, but in character-determining factors within the chromosomes. Jeffrey (18) is of the opinion that the

origin of new species is to be attributed to hybridization between species, although he says that it is "impossible to regard hybridization as the universal and sole cause of the appearance of new species." Other workers, of whom the most outstanding is de Vries (42), regard mutation as the cause of new species. It is generally conceded that the annual variety of *Melilotus alba* has originated from the biennial variety, but as to whether its origin is to be attributed to hybridization of the biennial variety with another species or to mutation within the biennial itself, the writer's investigations have thus far not produced evidence to warrant the drawing of any conclusions.

The furrowing process in *Melilotus* is especially noteworthy, for the formation of the furrows by vacuolation has not been previously described in the formation of microspores. From a careful study of the process it seems evident that there is a movement of granular cytoplasmic material toward the nuclei from the regions of the protoplast, equidistant between the nuclei. Thus hyaline areas are formed, and there is a higher surface tension in the vicinity of these areas than around the nuclei. There is an extrusion of liquid into vacuoles, which soon fuse, eventually forming rows of large vacuoles which would be continuous furrows but for the few strands of cytoplasm between them. The furrows which progress inward from the periphery until they meet the vacuoles are the result of the higher surface tension in these regions. The furrowing process may not be due to the same causes in all organisms, but from the study of the writer's material and from a study of the careful work of Bütschli, McClendon, Spek, Chambers, and Kite, the writer would conclude that the furrows which proceed from the periphery of the protoplast are to be attributed to the higher surface tension in the equatorial regions, whereas the cleavage of the cytoplasm in the deeper regions of the cell is caused by the extrusion of liquid into vacuoles which fuse, forming furrows.

The occasional formation of but a single pollen grain from each mother cell is quite unusual, and the only cases reported are those for the Cyperaceae which have been mentioned. *Melilotus* differs from *Carex*, described by Juel, in that a new wall is formed around the single microspore, whereas in *Carex* the old mother-cell wall becomes the microspore wall directly. The writer has not observed the meiotic divisions in abnormal pollen-formation in *Melilotus*, but both divisions occur, for in the abnormal pollen grains four nuclei may be seen. Although there may be irregularities in the heterotypic or the homoeotypic division in the mother cell, irregularities certainly exist previous to these divisions, as evidenced by the appearance of the mother cells while in the resting stage, for they are then very abnormal in appearance, particularly in being almost devoid of chromatin. Abnormality occurs, therefore, farther back in the life history of the plant than the meiotic division. The writer is inclined to regard this abnormality as due to hybridity between the annual and the biennial varieties. Further evidence that irregularity in pollen-grain formation is due to hybridity has been given by Jeffrey (18) for a number of plants in which it seems that

pollen abnormalities are unquestionably due to hybridity, particularly in species of *Potamogeton*, *Rubus*, and *Ranunculus*; and Longley (22), from his investigations on the genus *Rubus*, concludes that "multiplication of species in this genus has taken place by hybridization in their natural habitats."

### CONCLUSIONS

The evidence secured from a careful study of the cytological phenomena in microsporogenesis in the annual and biennial varieties of *Melilotus alba* indicates that the two varieties are identical cytologically, although they are functionally distinct. The evidence at hand warrants no conclusion as to the exact method of the origin of the annual variety.

The development of the anther is similar to the usual method of anther-development, with the exception that the pollen mother cells in each anther lobe are derived from a single row of cells of the primary sporogenous layer rather than from the whole layer.

In both varieties the haploid number of chromosomes is eight, the diploid sixteen.

The daughter nuclei at the end of the heterotypic division are completely reorganized before the homoeotypic division is initiated.

Quadrupartition of the pollen mother cell is effected by means of furrows which are formed largely by a system of vacuoles that are met by ingrowing surface furrows, advancing centripetally only a short distance before cutting into the vacuoles. There is at no time any evidence of the formation of cell plates across the equators of the spindles. The process is not unlike that described for certain fungi, although it has not previously been reported in microsporogenesis in the higher plants.

During the early stages of nuclear development a massive callose wall is secreted by the protoplast of the pollen mother cell. As cleavage is initiated, the special wall, composed of dense refractive callose, is secreted around the protoplast just inside the first callose wall. As the cleavage furrows are nearing completion, the special wall advances centripetally with the furrows which soon cut into the vacuoles. Partitions, which are continuations of the incoming callose special wall are formed between the young microspores. These partitions are formed by the deposition of a callose secretion of the protoplasts on the surface of this incoming wall.

Giant pollen grains, each containing four nuclei, are found in both varieties, each pollen grain being the entire product of a single mother cell in which cell division has failed to occur to form the tetrad.

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### EXPLANATION OF FIGURES

All figures highly magnified but not drawn to the same scale.

#### PLATE XXV

FIG. 1. Median section of nucleus in resting stage.  $\times 1500$ .

FIG. 2. Leptonema, median section. Nucleolus differentiated into two regions.  $\times 1500$ .

FIG. 3. Synizesis. Chromatin massed at one side of the nucleus. Nucleolus shows a bud being constricted off. Callose mother-cell wall being secreted at corners of the protoplast.  $\times 1400$ .

FIG. 4. The anastomosing threads are seen as the nucleus comes out of synizesis. The callose mother-cell wall partly formed.  $\times 1400$ .

FIG. 5. Pachynema. Threads much thicker. Nucleus is still seen. Callose mother-cell wall fully formed.  $\times 1400$ .

FIG. 6. Chromatin thread has broken up into a number of irregular pieces.  $\times 1400$ .

FIG. 7. Median view of nucleus at diakinesis, showing eight bivalent chromosomes and the nucleolus which is differentiated into two regions.  $\times 1400$ .

FIG. 8. Side view of heterotypic metaphase, showing the chromosomes.  $\times 1500$ .

FIG. 9. Polar view of the heterotypic metaphase showing the eight bivalent chromosomes, which are easily seen to be double.  $\times 1300$ .

FIG. 10. Side view of heterotypic anaphase. Chromosomes closely massed together. Part of the callose mother-cell wall is shown.  $\times 1400$ .

## PLATE XXVI

FIG. 11. Side view of heterotypic telophase. Chromosomes distinct, and some are seen to be partly split. Eight univalent chromosomes are easily seen in the lower nucleus. Two centrosome-like bodies are seen.  $\times 1500$ .

FIG. 12. Nucleus of late heterotypic telophase. Chromosomes have fused.  $\times 1600$ .

FIG. 13. Resting stage of nucleus at end of heterotypic division. Nucleus completely reorganized.  $\times 1700$ .

FIG. 14. Median section of nucleus just previous to the initiation of the homoeotypic division. The chromosomes are split at the ends. Nucleolus is still evident.  $\times 1700$ .

FIG. 15. Median section of homoeotypic metaphase. Polar view of one spindle shows eight chromosomes. Spindles are nearly at right angles.  $\times 1500$ .

FIG. 16. Telophase of homoeotypic division. Three of the four nuclei are seen. In one nucleus the eight chromosomes can easily be counted. Three of the four centrosome-like bodies are shown.  $\times 1500$ .

FIG. 17. Nuclei in resting stage. Spindles have almost disappeared. Protoplast has begun to invaginate at the periphery at points equidistant from the nuclei. First evidence of special wall shown around the protoplast. Outside of this a part of the mother-cell wall is shown.  $\times 1500$ .

FIG. 18. Vacuoles have been formed in rows equidistant from the nuclei by the fusion of smaller vacuoles. Shallow furrow evident at top of figure. Many of the original small vacuoles are seen.  $\times 1500$ .

FIG. 19. Large vacuoles formed by fusion of smaller ones. Only a few strands of cytoplasm connect the future microspores. Cytoplasm has become denser around the nuclei. Special wall has thickened slightly.  $\times 1500$ .

FIG. 20. The few connecting strands of cytoplasm seen in figure 19 have been severed, forming continuous furrows. Special wall has grown into the furrows. Many small vacuoles are seen.  $\times 1500$ .

## PLATE XXVII

FIG. 21. Special walls have met in center, forming partition walls between the microspores. Special wall thickens. Mother-cell wall shown in part.  $\times 1500$ .

FIG. 22. Special wall complete. Cytoplasm of microspores more compact. Part of mother-cell wall shown.  $\times 1500$ .

FIG. 23. View of callose mother-cell wall and special wall drawn from living material.  $\times 400$ .

FIG. 24. Young pollen grain. Exine just forming. Two pores are shown.  $\times 1200$ .

FIG. 25. Mature pollen grain showing thick exine and generative cell.  $\times 1000$ .

FIG. 26. Transverse section of mature pollen grain. The three grooves are evident. Intine also seen.  $\times 1000$ .

Figures 27-33 are not drawn in proportion to actual size as compared with the other figures. These figures represent abnormal stages.

FIG. 27. Abnormal pollen mother cell which is to give rise to a giant pollen grain. Scant amount of chromatin in nucleus.  $\times 800$ .

FIG. 28. Abnormal pollen mother cell at end of homoeotypic division. Three of the four nuclei shown. Protoplast has failed to round up.  $\times 700$ .

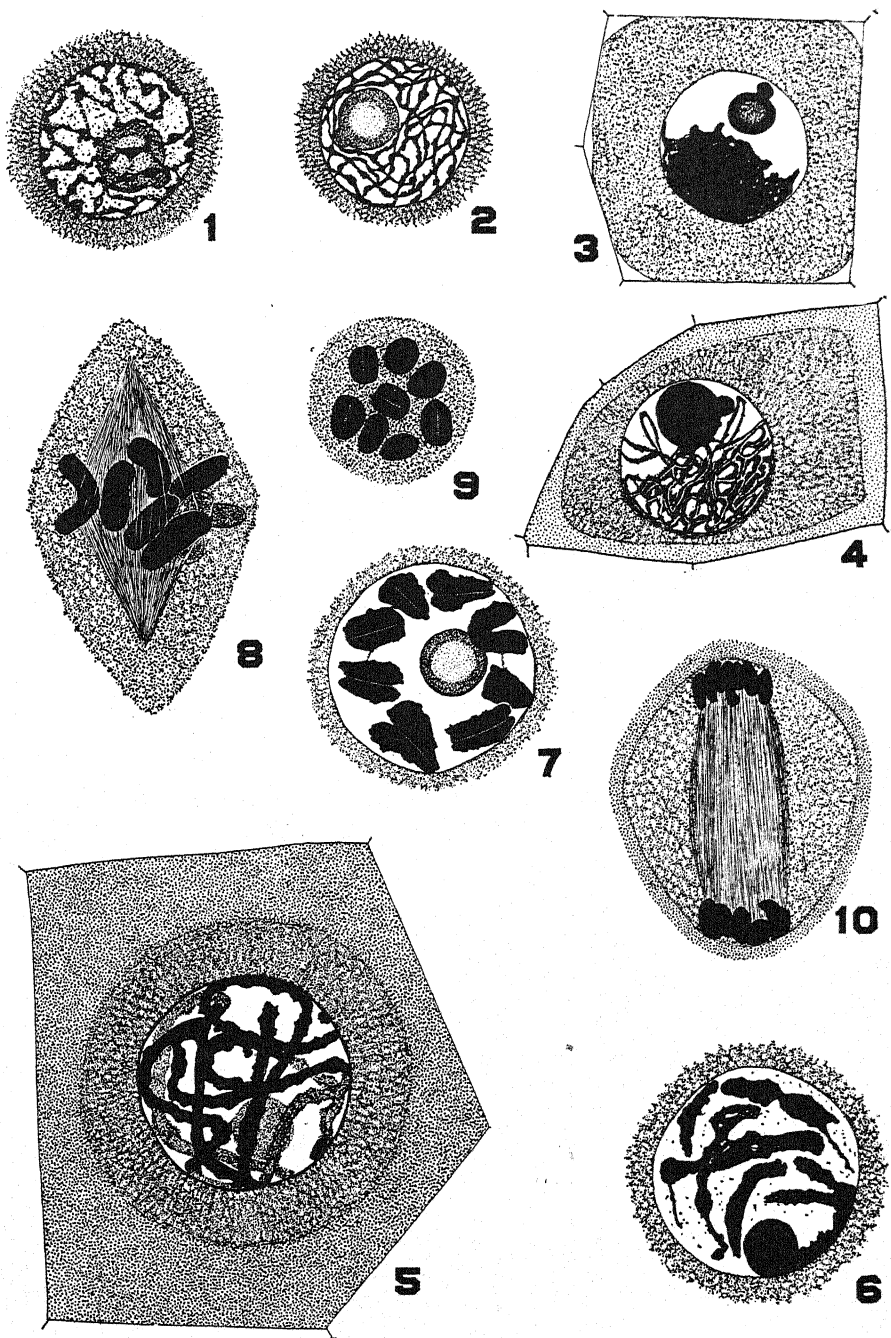
FIG. 29. Tetrad from abnormal mother cell. Microspores have failed to separate. The wall between them is exine.  $\times 300$ .

FIG. 30. Pollen mother cell has formed only two microspores by a single cross wall.  $\times 300$ .

FIG. 31. Giant pollen grain containing four nuclei. No pores in exine.  $\times 300$ .

FIG. 32. Giant pollen grain with generative cell.  $\times 300$ .

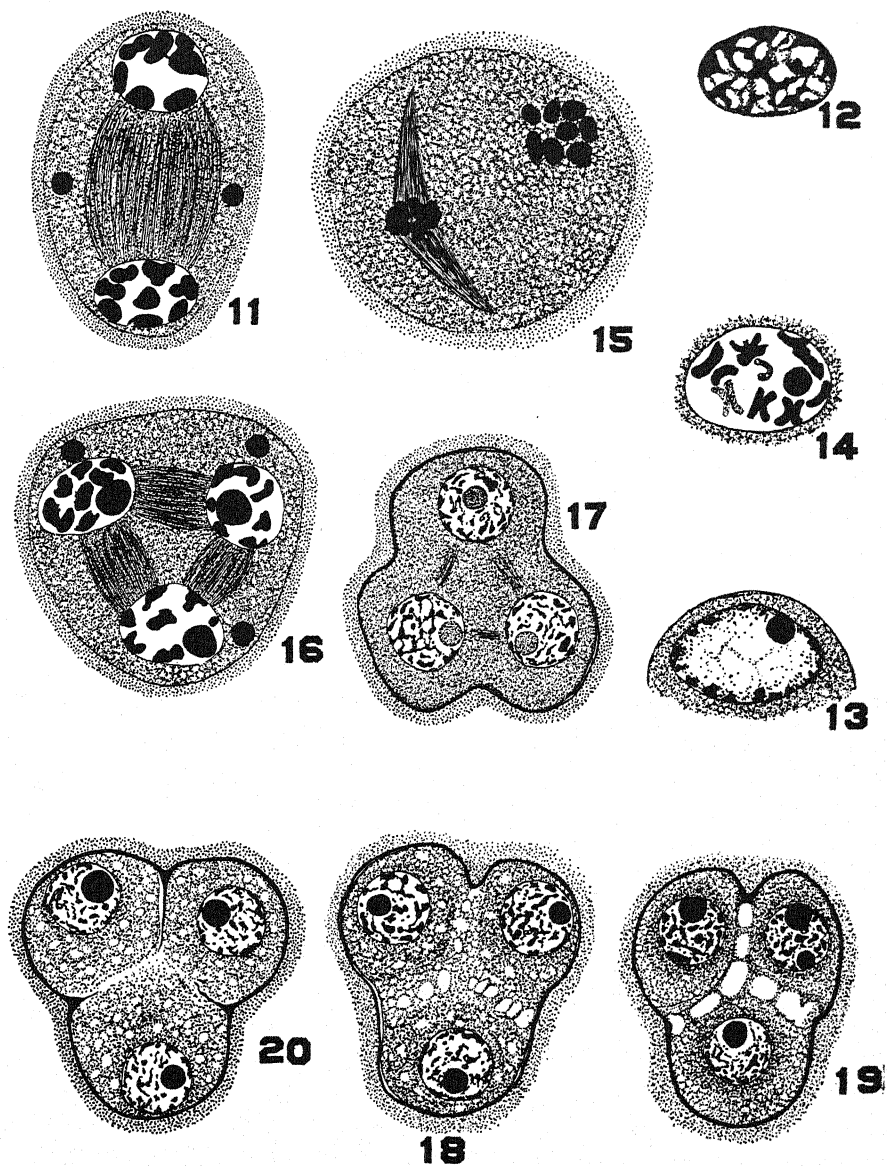
FIG. 33. Giant pollen grain in which the four nuclei have fused in pairs.  $\times 300$ .



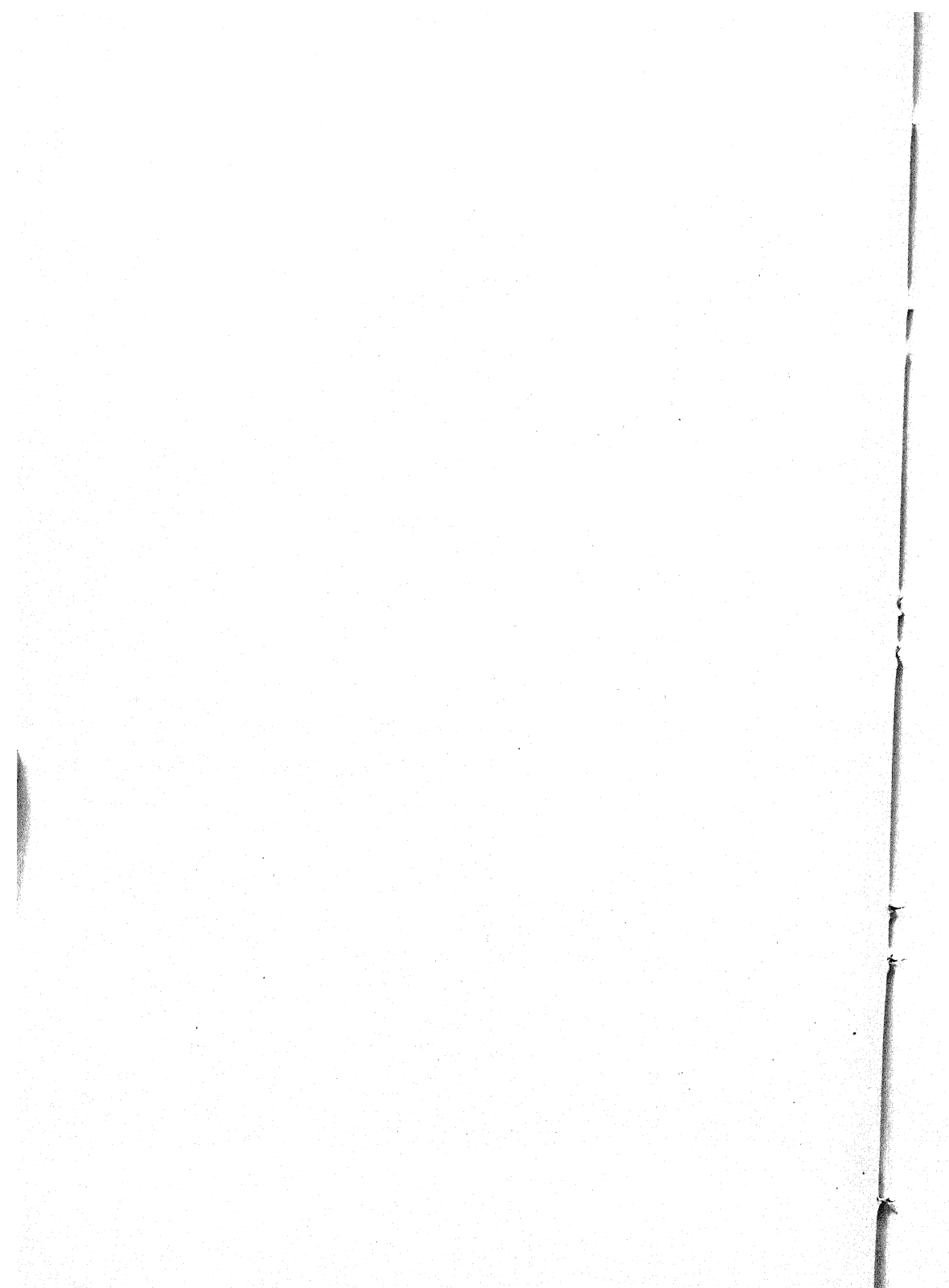
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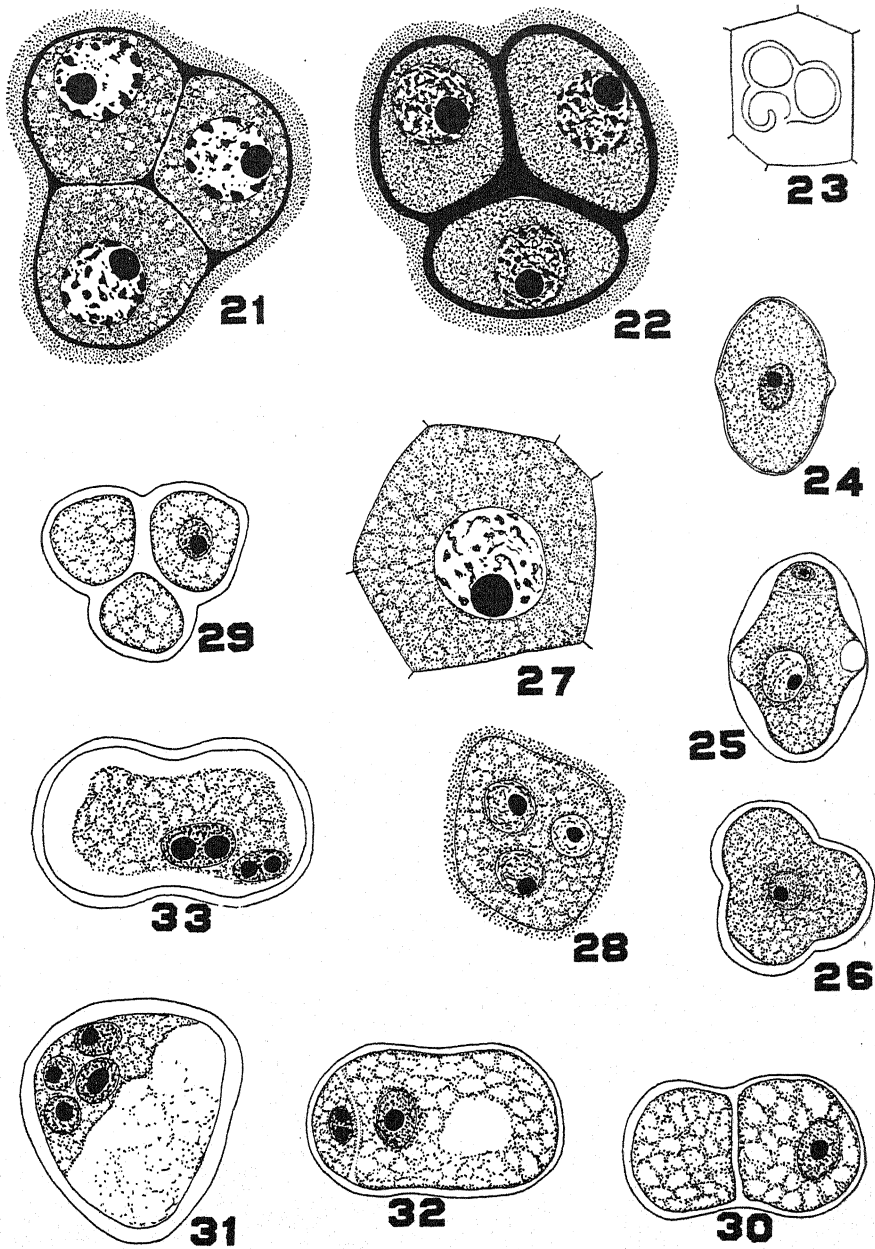






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## THE INHIBITION OF BUD-DEVELOPMENT AS CORRELATED WITH THE OSMOTIC CONCENTRATION OF SAP

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Commonly during the growing season only a few of the many buds of the majority of plants ever develop into shoots, though practically all the buds, excepting flower buds, are potentially capable of producing shoots. In many plants the apical or terminal bud is the only one to develop, or if it develops more strongly than the others it seems to inhibit partially or almost completely the bud below it, for, when it is removed, one or more of the subapical buds begin to grow. This dominance by the apical bud may persist throughout the life of the plant. On the other hand, in some plants the lateral buds may grow vigorously, while in still other cases the apical bud may be strongest only during certain periods of the life of the plant and its dominance may be very easily altered. The above-described phenomena have been familiar for a long time to all students of plant growth, and, of various hypotheses offered to explain them, one that has been strongly emphasized among recently published papers (Loeb, 20; Reed, 24) is that the apical shoot produces some sorts of "inhibiting substances" which move toward the base, preventing the development of the more basal buds. "Growth-promoting substances" and "hormones" have also been suggested as possible causes of this behavior (Reed and Halma, 25).

In some experiments with cuttings of *Ligustrum*, Curtis (11) found that, though normally only the upper buds of cuttings developed shoots, when the twigs were placed with their bases in strong sugar solutions the lower buds developed and the upper buds remained dormant. Later, in some preliminary tests, Curtis (12) found "that the upper part of a rapidly growing shoot may have a concentration that would give a pressure over two atmospheres greater than the concentration in the middle or lower part of the same shoot." He goes on to suggest that, when water becomes deficient, the tissue near the apex, if it has the greater osmotic concentration, will tend to be more successful in the competition for this water than will other tissues with lower osmotic concentrations. Terminal buds will therefore grow and apparently inhibit the growth of the lower buds.

It has long been recognized that the consideration of the water relations of plants is of fundamental importance. If the amount of water available

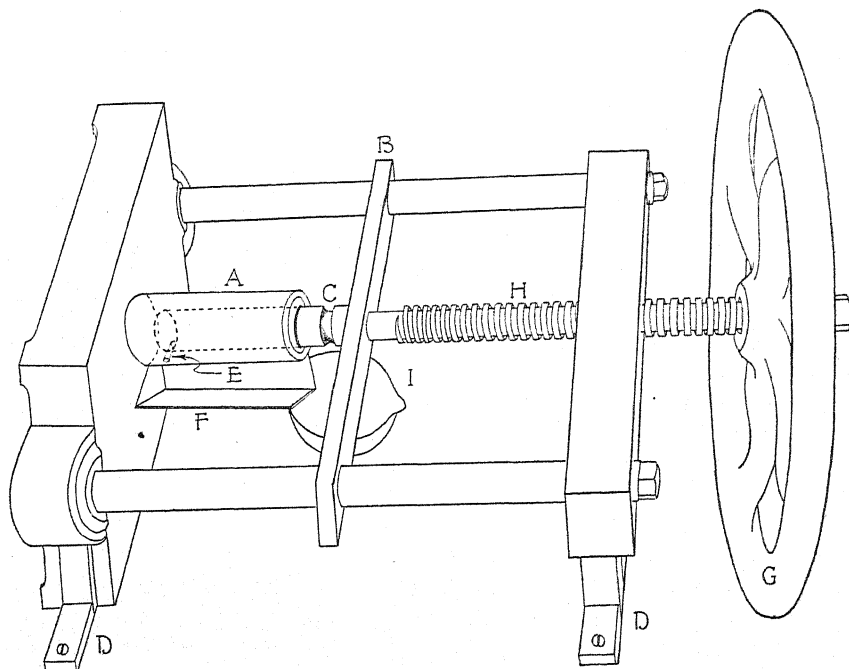
is important to the life, development, and activities of the plant as a whole, it is certainly valuable for the different organs of the plant and primarily for the individual cells of those organs. The external factors will be treated only incidentally, since this study is concerned with portions of plants. In addition to colloidal hydration and dehydration, hydrogen-ion concentration, and various chemical and physical changes taking place within the cell, the osmotic concentration of the cell sap, either alone or in combination with one or more other factors, is commonly the most important internal factor which may influence the amount of water readily accessible for the protoplasm. Growth and division take place only in those cells which are turgid. Assuming the presence of a semi-permeable membrane, the two most important factors that produce turgidity are a high concentration of the solutes and a ready supply of the solvent. Other things being equal, that part of a plant will grow most strongly which can, on account of osmotically active substances, compete successfully with the rest of the plant for water. Perhaps one of the clearest demonstrations of this competition for water between parts is shown in the experiments of Chandler (9). He observed that with two tissues of different osmotic concentrations grafted together, when water became deficient, the one with the higher osmotic concentration withdrew water from the other and thus caused it to wilt. Ordinarily fruit trees have such a supply of water that there is no apparent competition between the different parts, but in cases of extreme drought the leaves which have a higher osmotic concentration may actually withdraw water from the green fruit which has a lower osmotic concentration and thus cause it to wither.

Therefore, to see if there were any correlation between bud-development and osmotic concentration of the sap, series of freezing-point determinations were made of sap that had been extracted by pressure from different portions of stems and of potato tubers. A comparison of the freezing-point depressions is practically the same as a comparison of the osmotic pressures, since the solutions were relatively dilute.

#### PROCEDURE AND METHOD

The method used for determining the concentration of the sap was essentially that described by Harris and Gortner (15)—namely, first to render the tissue more permeable by freezing in an ice-salt mixture; then to extract the sap by pressure and to determine its freezing-point with a Beckmann thermometer. In addition to potato tubers, stems of woody plants, such as Philadelphus (*Philadelphus pubescens* Loisel.), privet (*Ligustrum ovalifolium* Hassk.), chestnut (*Castanea dentata* Borkh.), and grape (*Vitis labrusca* L.), and in some cases succulent stems of Bryophyllum (*Bryophyllum calycinum* Salisb. = *B. pinnatum* Kurz.), tobacco (*Nicotiana* sp.), and tomato (*Lycopersicon esculentum* Mill.) were used. Every precaution to avoid loss of water by evaporation was taken. In the laboratory

the material was placed for at least ten hours in large test tubes in an ice-salt mixture which maintained a temperature range of  $-5^{\circ}$  to  $-15^{\circ}$  C. The press used was of the type ordinarily employed in laboratories for extraction purposes, but placed horizontally (see text fig. 1). In it was placed a thick-walled metal cylinder, *A*, having a length of 9.6 cm. and an



TEXT FIG. 1. Apparatus used for expressing sap.

internal diameter of 3.2 cm. and walls 0.9 cm. thick. It is closed at one end, and into the other is fitted a removable solid piston, 10.5 cm. in length and 3.2 cm. in diameter. Near the base of the inside of the cylinder is an opening, *E*, 3 mm. in diameter, which provides an outlet for the expressed fluids. To retain the solid matter in the press a small plug of cotton is placed on the inside over this opening. Although the piston, *C*, fits so tightly that when the opening is closed and the cylinder inverted the piston does not fall out, yet, when pressure is applied to the tissue in the cylinder, liquid not only flows out of *E* but also out of the top of the cylinder. To obviate this difficulty the press was placed horizontally as in the diagram, and a groove was cut around the end of the cylinder to direct the flow of sap. A paraffined trough, *F*, placed under the cylinder, collects the sap from both places and directs its flow into the dish, *I*. This arrangement provides for a minimum loss of sap due to evaporation and to wetting of surfaces.

This press is practically a jack-screw placed horizontally, and therefore its efficiency may range from 10 percent to 50 percent. Now, since

$$\text{efficiency} = \frac{\text{work out}}{\text{work in}},$$

and

$$\text{work out} = W (\text{force exerted}) \times p (\text{pitch of the screw}),$$

then

$$\text{efficiency} = \frac{W \times p}{F \times 2r} \quad \text{or} \quad W = \frac{\text{efficiency} \times (F \times 2r)}{p}.$$

Assuming the lowest efficiency of 10 percent, and the smallest force exerted on the outside of the wheel,  $G$ , as 100 pounds, and since the radius of  $G = 7.5$  in. and the pitch of  $H = 0.25$  in., then

$$W = \frac{.10 \times (100 \text{ lbs.} \times 2\pi \times 7.5 \text{ in.})}{.25 \text{ in.}} = 1,880 \text{ lbs.}$$

Since the area of the inner end of the piston,  $C$ , is 1.22 sq. in., the amount of stress per square inch on the tissue will be at least 1,540 lbs. This is a very conservative estimate. The data of Knudson and Ginsberg (18) demonstrate that great changes in pressure decidedly influence the concentration of the expressed sap. The results of comparative tests made with the apparatus described by these authors and with the one described above are given in table 1 below. With the cylinder used by them in an hydraulic press,<sup>1</sup> it was possible to obtain the largest pressure recorded by Knudson and Ginsberg, namely, a total pressure of 50,000 lbs., or a pressure of 3,978 lbs. per sq. in. (diameter of piston = 4 in.). Extractions were made of similar material of *Philadelphus* stems on the same day, and the

TABLE 1. *Comparison of Tests of Sap from Different Portions of Philadelphus Stems Made with the Apparatus Used by the Author and with the Apparatus Described by Knudson and Ginsberg (18)*

A			B		
Hand Press and Small Cylinder Used by Author			Hydraulic Press and Large Cylinder		
Calculated minimum pressure = 1,540 lbs. per sq. in. Actual pressure probably nearer 5,000 lbs. per sq. in.			Pressure = 3,978 lbs. per sq. in., the same as the maximum obtained by Knudson and Ginsberg		
Portion of Stem	$\Delta$	Percentage of H <sub>2</sub> O in Residue	Portion of Stem	$\Delta$	Percentage of H <sub>2</sub> O in Residue
1st 7 in.....	.98°	32.3	1st 7 in.....	.91°	48.2
2d 7 in.....	.74°	31.3	2d 7 in.....	.75°	51.6
3d 7 in.....	.65°	30.2	3d 7 in.....	.72°	48.1

<sup>1</sup> The hydraulic press was very kindly placed at the writer's disposal by the College of Engineering at Cornell University.



percentage of water remaining in the residue from the tissue after expression of the sap was determined. The freezing-point depressions of the sap show in each case similar relative values as between different parts of the stem. Since in every case the percentage of water is less with the smaller press, the apparatus used in this work expresses more sap from the tissues and doubtless develops a greater pressure per square inch. Thus both by experiment and by calculation the pressure on the tissue was not less than 1,540 lbs. and probably more than 4,000 lbs. per square inch. There is no doubt that in the majority of cases it was much greater and, as we shall see later, probably uniformly high.

In the preliminary work large differences were found in the freezing-point depressions of different tissues. To demonstrate that this result was not due to differences in pressure, a series of tests were made in which the percentage of water retained in the residue after the extraction was determined. This method was used since there was no practical method for measuring the exact pressure applied for each extraction. The results are given in table 2. Note particularly in the tests for April 6 that when  $\Delta$ , the depression of the freezing-point, varies greatly from the average, there is not enough variance in the percentage of water to explain the difference. Therefore differences in freezing-point depressions of sap in these cases were not due to differences in pressure.

TABLE 2. *To Show that Differences in Freezing-point Depression are not Due to Differences in the Amount of Water Extracted from Different Portions of Potato Tubers*

Date (1922)	Portion of Tuber	$\Delta$	Percentage of H <sub>2</sub> O
April 6.....	Sprout	1.180°	37.5
April 6.....	3 apical eyes	.780°	37.1
April 6.....	3 basal eyes	.710°	36.3
April 6.....	3 middle eyes	.740°	36.1
April 6.....	3 apical eyes	.955°	31.5
April 6.....	3 basal eyes	.880°	36.0
April 6.....	3 middle eyes	.970°	37.5
April 7.....	Sprout	1.210°	37.7
April 7.....	3 apical eyes	.860°	31.3

Also it seemed possible that in woody stems the press may have extracted more sap from the younger succulent portions than from the older woody portions because of the greater rigidity of the cell walls in the latter case. An examination of table 3 below shows that in all cases there is a gradual decrease in concentration of sap from the apical portions to the lower portions of the shoots. These differences are not due to differences in the amount of sap extracted, for in two cases the lower portions (third 7-in. portion) have a smaller percentage of water in the residue than in the apical portion (first 7-in. portion), and in all cases a lower percentage than in the portion directly above them (second 7-in. portion). Therefore the smaller freezing-point depression in the lower portions is not due to less efficiency

in the extraction of the sap. Furthermore, the differences in percentage of water in the residue can not account for the differences in freezing-point depressions. For, as shown in groups 4 and 6, where there is a relatively uniform percentage of water in the residue (group 6), the freezing-point depression gradient is practically the same as where the percentages of water in the residues show greater variation (group 4). This work is concerned with relative rather than with absolute values.

TABLE 3. *Comparison of the Percentages of H<sub>2</sub>O Left in Young Succulent Tissue with that of Older, more Woody Tissue of Philadelphus*

Group	Date (1922)	Portion	$\Delta$	Percentage of H <sub>2</sub> O
1.....	June 19	1st 7 in.	.860	49.1
	June 19	2d 7 in.	.670	59.6
	June 19	3d 7 in.	.480	47.4
2.....	June 19	1st 7 in.	.810	39.3
	June 19	2d 7 in.	.620	48.4
	June 19	3d 7 in.	.550	45.6
3.....	July 10	1st 7 in.	.940	34.8
	July 10	2d 7 in.	.769	45.9
	July 10	3d 7 in.	.590	43.3
4.....	July 10	1st 7 in.	.930	39.4
	July 10	2d 7 in.	.800	53.8
	July 10	3d 7 in.	.590	50.3
5.....	July 10	2d 7 in.	.610	52.9
	July 10	3d 7 in.	.560	48.5
6.....	July 20	1st 7 in.	.980	32.3
	July 20	2d 7 in.	.740	31.2
	July 20	3d 7 in.	.650	30.2

The sap was stored in a cool place, and the freezing-point determinations were made within a few hours because changes take place when the sap is stored for any length of time. In some cases there was an increase of 0.104° C. and in other cases a decrease of 0.015° C. in the depression of sap that had been in the refrigerator 24 hours. Only rarely was there no change. On this account, throughout each series special care was taken to make determinations with sap that had been handled under identical conditions and never more than two hours after extraction. The diameter of the test tubes was such that a 2-cc. sample of sap was sufficient to cover the bulb of the thermometer. The freezing-point of water taken in many cases at the beginning and end of a series of determinations showed practically no variation and rarely more than 0.01° C. The method of inoculation with a crystal to control the amount of undercooling was not followed here, since, on account of the small amount of sap used in a sample, it would introduce a source of error. The sap was allowed to undercool approximately one degree below its freezing-point, when a quick twist of the

thermometer was usually sufficient to produce ice-formation. Corrections for undercooling were made with the formula used by Harris and Gortner (15),  $\Delta = \Delta' - 0.0125\mu\Delta$  ( $\mu$  = the amount of undercooling for the freezing point  $\Delta'$ ).

#### PRELIMINARY STUDIES

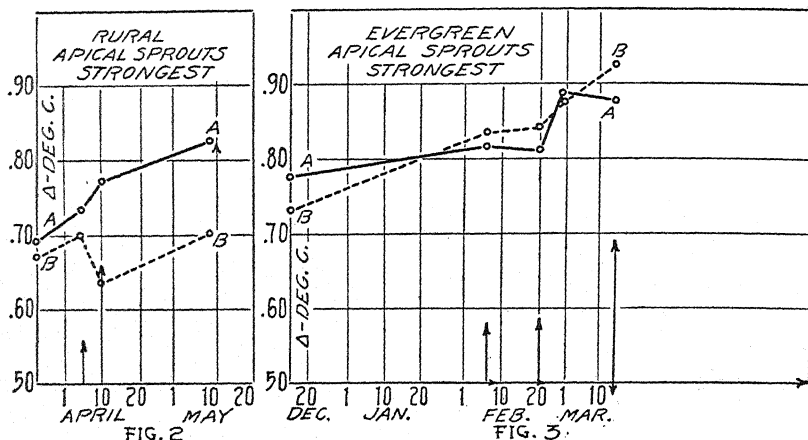
In the preliminary experiment *Bryophyllum* plants grown in the greenhouse were used. In this experiment only was the tissue ground in a mortar. So that the study might be limited to conditions in the stem only, the leaves were removed 48 hours before samples consisting of successive pairs of nodes from three portions, *A*, *B*, and *C*, of the stem were taken. In the two tests made when the plants were growing actively, the values for *A*, the apical portions of four stems combined, were greater in both sets than those of *B*, the portion below *A*, and in one case greater than *C*, the lowest portion. However, when the later tests were made the plants were growing slowly at the apex, and vigorous shoots started from the lowest nodes of the plants that were left; in only two tests out of a total of 15 was there a greater depression in the apical portion. In these plants, therefore, the apical portion did not grow strongly enough to inhibit the growth below, neither did it have a concentration uniformly greater than the lower portions. Thus, in these instances, when the concentrations in the apical portions did not exceed those of the lower portions, there was a corresponding failure of an inhibition of growth of the lower buds by the apical ones.

#### EXPERIMENTS WITH POTATO TUBERS

To obtain evidence from materials in which the apical buds were dominant, experiments were planned with potato tubers.<sup>2</sup> They were selected because they are reduced stems, and because growth of the buds on account of the large amount of stored food is very rapid and gives an abundance of growing material at a time when other stems are still dormant. In the tests, uniform plugs of tissue 10 × 13 mm. were removed at the eyes of the tubers with a cork-borer. Sample *A* was composed of tissue from the three eyes nearest the apical end of the tuber, and sample *B* of tissue from the three eyes nearest the basal or stem end. At intervals of a week or more, samples of sap from different potatoes of the same lot were tested. The average freezing-point depressions of the sap of *A*, the apical portion, on successive days were 0.694°, 0.766°, and 0.825°, and are represented by the solid line *AA* in text figure 2, while the average freezing-point depressions of the sap of *B*, the basal portions, were 0.671°, 0.700°, 0.638°, and 0.700°, and are represented by the broken line *BB*, text figure 2. On the last three dates the sprouts which had developed were included in the

<sup>2</sup> The different varieties of tubers used in these experiments were identified. Many tubers were furnished by the Department of Vegetable Gardening of the New York State College of Agriculture.

samples. For the relative length of the sprouts on any date see the arrows at the bottom of the figure. On all these dates the eyes near the apex had a greater average concentration of sap than the eyes near the base. When potatoes from this same lot were left and allowed to sprout, the eyes at the apical end developed sprouts while the others did not.



TEXT FIGS. 2, 3. Comparison of freezing-point depressions,  $\Delta$ , of the sap of the apical and basal portions of potato tubers on different dates. FIG. 2. Comparison of the sap from the apical portions of those potato tubers which develop strong apical sprouts (Rural type) with the sap from the basal portions of the same tubers. FIG. 3. Comparison of the sap from the apical portions of those potato tubers which do not develop strong apical sprouts (Evergreen variety) with the sap from basal portions of the same tubers.

Solid line AA,  $\Delta$  of sap of 3 eyes nearest apical end.

Broken line BB,  $\Delta$  of sap of 3 eyes nearest basal end.

Arrows above X axis indicate relative length of sprouts from the apical portion.

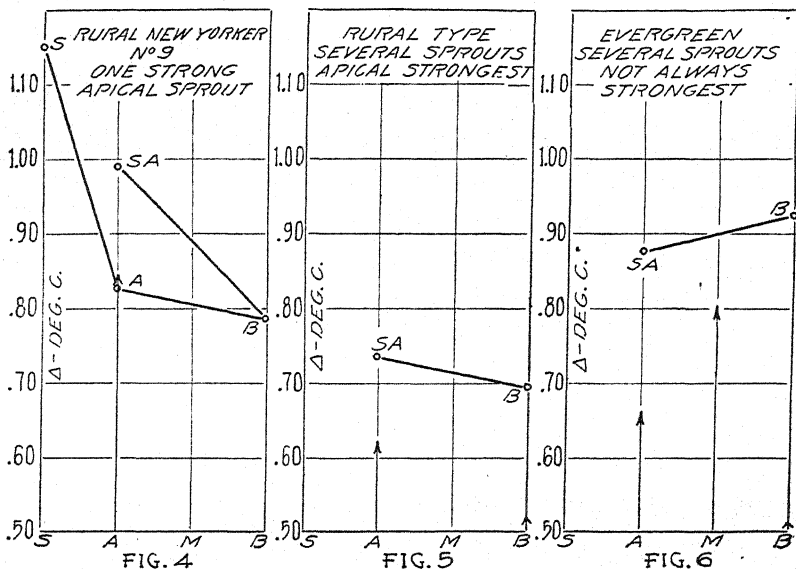
Arrows below X axis indicate relative length of sprouts from basal portion.

Arrows on X axis indicate relative length of sprouts from middle portion.

In a second lot of potatoes in which the apical eyes did not always develop sprouts more strongly than the basal eyes, we have the values for the freezing-point depressions of the apical portions, A, represented by the solid line in text figure 3, and the values for the freezing-point depressions of the basal portions, B, by the broken line. A comparison of the relative length of the sprouts, which may develop at any portion of the tuber (see arrows at the bottom of the figure), with the two curves mentioned above shows that when there is not always a greater depression of the freezing-point at the apical end as compared with that at the basal end, there is a corresponding irregularity in the sprouting.

In a strain of Rural New Yorker, variety no. 9, under typical storage conditions, a strong sprout develops at the apex and none develops at the other eyes. In the five tests taken, the sprouts had an average freezing-point depression of  $1.150^{\circ}$ , the three apical eyes had an average depression

of  $0.826^\circ$ , and the three basal eyes had an average depression of  $0.788^\circ$ . These values are represented graphically by *S*, *A*, and *B* respectively in text figure 4 and show that the sprout or actively growing portion had a decidedly greater concentration than any other portion. The average of the values for *S* and *A* gives a value of  $.988^\circ$ , and the solid line between the point *SA* (this average) and *B* illustrates the extent of this difference between the two ends of the potato when the sprouts are included with the tissues at the eyes. This is shown further if we plot two more curves,



TEXT FIGS. 4-6. The average values for the freezing-point depressions,  $\Delta$ , of the sap of different portions of potato tubers of three different types, having developed (1) one strong sprout at apical end (fig. 4, Rural New Yorker no. 9), (2) several sprouts, apical sprout being strongest (fig. 5, Rural type), (3) several sprouts, apical sprout not always strongest (fig. 6, Evergreen).

*S*,  $\Delta$  of sprouts.

*SA*,  $\Delta$  of sprouts and tissue at 3 eyes nearest apical end.

*A*,  $\Delta$  of tissue at 3 eyes nearest apical end.

*B*,  $\Delta$  of tissue at 3 eyes nearest basal end.

Arrows at *A* indicate relative length of sprouts at apical portion.

Arrows at *B* indicate relative length of sprouts at basal portion.

Arrows at *M* indicate relative length of sprouts in middle portion.

using, in text figure 5,  $0.735^\circ$  for *SA*, the average of the values for the three apical eyes and sprouts after developing sprouts, and  $0.700^\circ$  for *B*, the average of the values for the three basal eyes and sprouts from text figure 2, and in text figure 6 using from text figure 3 the March 14 values when the potatoes were at a corresponding stage. Comparing the curves, *SA-B*, of text figures 4, 5, and 6, it becomes evident that, in text figure 6, in potatoes

in which the apical eyes are not the only ones to develop sprouts, the apical portion has usually a lower concentration than the basal portion, while, when the sprouts of the apical eyes develop but not especially more strongly than those of the basal eyes, there is a slightly higher concentration in the apical eyes, as is shown in text figure 5. Furthermore, when there is a very strong apical sprout there is a decidedly greater concentration in this sprout than in the tissue of the tuber at the apical end, as in text figure 4, *SA-B*. This great difference is not due entirely to the sap of the sprouts, because *A*, the value of  $\Delta$  for the apical end when the sprouts were not included, is greater than *B*, the corresponding value of  $\Delta$  for the basal portion. Thus, in this earlier work, tubers of potato in which there was a strong terminal sprout always had a decidedly greater concentration in the sprout than in any portion of the tuber, and with but few exceptions a greater concentration in the apical eyes; whereas, tubers which sprouted irregularly showed only occasionally a slightly greater concentration in the apical eyes than in the basal eyes. This evidence seems to indicate that there was some correlation between the osmotic concentration of the sap of different portions of the tuber and the way in which the sprouts developed.

Thus in these experiments with potatoes it was found that on four different dates from March 23 to May 10 tubers from a lot (Rural type, 1920) which developed the strongest sprouts in the eyes at the apical end, always had a greater depression of the freezing-point of sap of the tissue from the eyes nearest the apical end; whereas, of tubers from another lot (Evergreen, 1920), which developed sprouts irregularly, those sprouts at the apex, not always being the strongest, only occasionally had a greater freezing-point depression of sap at the apical end.

These tests with three types of tubers at parallel stages of development as to sprouting show that one lot (Rural New Yorker no. 9), having a single strong sprout at the apical end, gave a decidedly greater depression of the freezing-point of the sap from the three eyes (sprouts included) nearest the apical end; also that a second lot (Rural type, 1920) having several sprouts, of which the apical sprout was the strongest, had a slightly greater depression of the freezing-point in the apical region (sprouts included); and a third lot (Evergreen, 1920), having several sprouts of which the apical sprout was not always the strongest, had a slightly smaller depression of the freezing-point at the apical end (sprouts included).

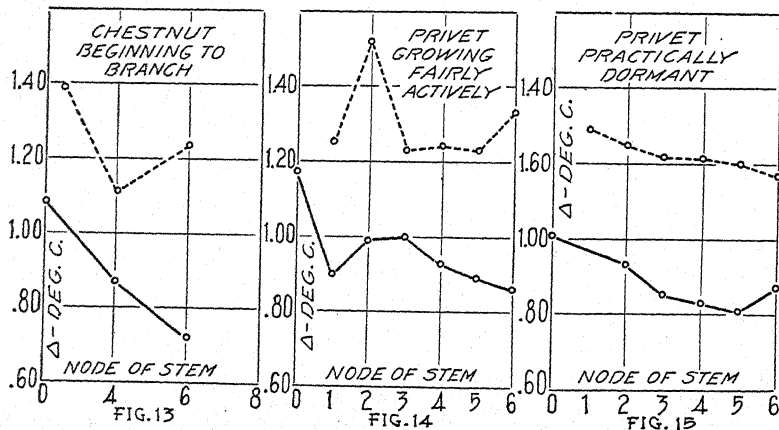
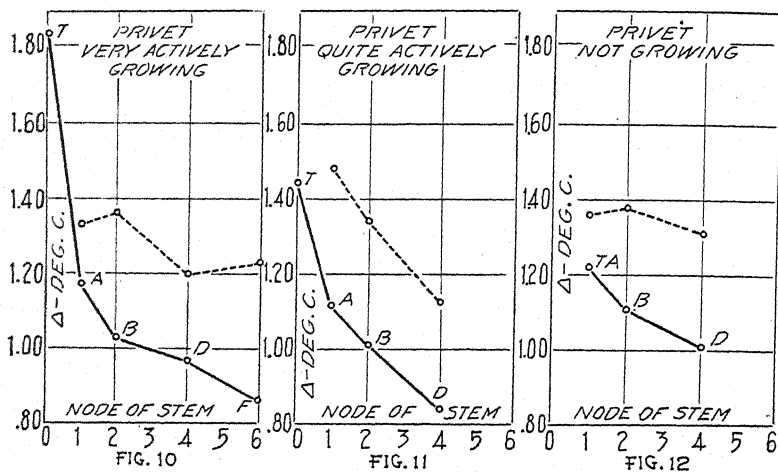
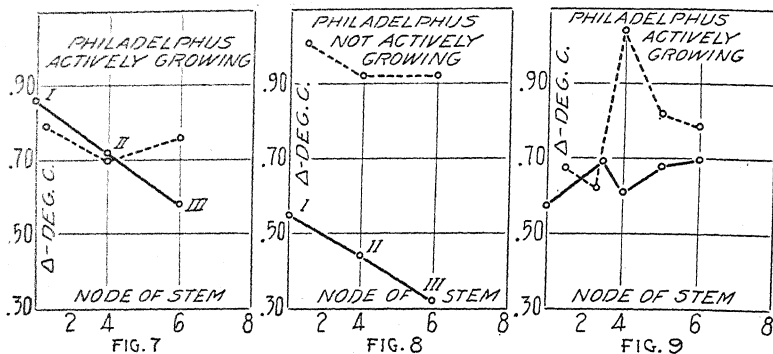
It is evident that the so-called varietal differences in degree of polarity of tubers, as indicated by the method of development of the sprouts, may be upset by environmental conditions, because in other tests exposure to low temperatures (approximately 0° C.) in February, before the sprouts began to appear, resulted in potatoes, which ordinarily developed a single strong apical sprout, producing strong sprouts also in other regions. Moreover, the relative values for the freezing-point depressions of the apical and basal portions were altered so that there were no distinguishing differences.

In another lot of tubers, infection caused changes which modified the osmotic conditions in the tubers. Before the tubers were tested they had been transferred from a moderately cool and moist cellar to the warmer and drier atmosphere of the laboratory. In other cases these changes altered the sprouting so that the terminal sprouts were stronger. Both the freezing-point depressions of the sap and the manner of development of the sprouts were affected by the degree of maturity as produced by differences in the duration and season of the growing period. The freezing-point depression fluctuated considerably during storage. Just before the sprouts began to appear the sap of the tubers gave an increased depression of the freezing point, and after the sprouts began to develop there was a decrease. In all cases the sap of sprouts gave a decidedly greater freezing-point depression than that of other tissues..

#### EXPERIMENTS WITH STEMS

For three summers, in seven sets of tests with *Philadelphus* twigs, when freezing-point depressions of the sap from successive 20-cm. portions of several actively growing shoots were determined, the portions nearest the terminal end had a decidedly greater concentration in every case than those lower. The solid line curve, I-II-III, of text figure 7 is typical. Similar results were obtained with privet shoots, and the solid lines of text figures 10 and 11 are representative. Where the growth of the shoots was exceptionally active we find a very much higher concentration in the terminal portion and a steeper gradient. (In text figure 10 compare the value for *T*, the terminal portion, with those for the lower portions, *A*, *B*, *D*, and *F*.) On the other hand, in two trials with twigs which were not growing at the apex, although the terminal portion was included with the sap of the first node as shown in the curves of text figure 12, we do not find so steep a gradient as in the solid-line curves of text figures 10 and 11.

During the earlier work with *Philadelphus* it was not possible to test shoots which had stopped elongating, but later the sap of shoots in which the terminal portion, as well as the rest of the stem, had practically ceased growing was found to have a greater concentration in the upper and terminal portions than in the lower. This is represented by the points I, II, and III in text figure 8. Moreover, chestnut twigs in which longitudinal growth had ceased and which were beginning to branch gave in the solid line of text figure 13 a similar gradient. If we analyze the possible effect of the leaves on the ability of the stem to grow, or to command an abundant supply, we see that shoots of *Philadelphus* (text fig. 8) and of privet (text figs. 12 and 15) which are not growing actively always have decidedly greater values for the leaves (broken line) than for the corresponding portions of the stems (solid line). When the stem is growing (see text figs. 7, 9, and 10) this is not always true, and the concentration of the sap of the apical portion more nearly approaches and in two cases exceeds that



TEXT FIGS. 7-15. Comparison of freezing-point depressions,  $\Delta$ , of different portions of stems and of leaves from different nodes of various woody plants under different conditions of growth.

Solid line,  $\Delta$  of portions of stems.

Broken line,  $\Delta$  of leaves.



of the leaves. This is most strikingly shown in privet (text fig. 10) when the sap of the very actively growing terminal portion gives a depression of the freezing-point of over two tenths of a degree more than the leaves of the first node, while, when the terminal portion is not growing as actively (text fig. 11), the leaves of the first node have a slightly greater concentration of sap and the second node leaves have only a slightly smaller concentration.

The curve of text figure 9 was not typical, probably because there were unusual weather conditions before the material was collected. For several days it was very cloudy and relatively cool, with a high relative humidity. On the other hand, the sap of the chestnut leaves, which were growing in bright sunlight, always had a greater depression of the freezing-point. In privet (text fig. 12), when the stem had practically ceased growing the leaves of each portion had always a greater concentration than the stem. When, on the other hand, active growth was continuous, the sap of the leaves of the first nodal portions in privet usually gave a smaller depression of the freezing-point than the sap of the tip or the first node, and if higher, as in text figures 9, 10, and 11, the difference was not significant for the data presented. Text figure 15 gives the results of similar determinations made from 100 twigs growing on the same hedge but which because of their position had practically ceased growing. Similar results in several instances were observed with *Philadelphus*, of which text figure 8 is representative. If the curves mentioned above are compared with those of text figures 9 and 10, it is evident that when growth is taking place the same stem gives not only more differences in value between the different portions but greater differences.

The objection may be offered that the greater freezing-point depression of the sap from the apical portion of the stem, as indicated in text figure 7, may have been due to the addition of the sap from the small leaves in the unfolding terminal bud which may have a greater concentration of sap. In actively growing shoots of *Philadelphus*, however, the sap of the leaves of the upper portion frequently has a smaller depression of the freezing-point than does the sap of the stems, as illustrated in the leaf and stem curves of text figure 7. In a further study, 100 uniform shoots on the same privet hedge which extended in a north and south direction were selected. On September 4, twenty were cut; freezing-point depressions of samples of sap of the stem and leaves of the first three nodes were  $1.215^{\circ}$  and  $1.250^{\circ}$  respectively, and depressions of the next three nodes below were  $0.890^{\circ}$  and  $1.349^{\circ}$  respectively. On this same date the unfolding terminal bud was removed from forty shoots, and forty more were left for comparison. On two later dates determinations of sap of portions of twenty shoots from each of these lots were made. On September 6, the value,  $1.017^{\circ}$ , for the upper portion of the stem, which in this case included the bud with its leaves, is smaller than the value,  $1.209^{\circ}$ , for the stem when the tip was not included.

On September 12, there is a value of  $0.993^{\circ}$  as compared with  $1.089^{\circ}$  in the second case. Evidently the lower portions (nodes 4-6) were not affected by the removal of the tip, for in both sets the curves are practically the same.

In order to show that the smaller values for the lower portions of the stem were not due to a dilution of the sap from the greater amount of sap in the internodes, tests were made of samples of sap taken separately from the regions of the node and from the internodes. In several series of tests the sap from the internodes was not always more dilute than the sap from the nodal region on either end, and when it was less concentrated the difference was not significant.

A few determinations were also made with samples of sap from the stem inside the cambium of more mature portions of *Philadelphus* shoots and with samples from the region outside the cambium. Since the outer portion gave the value of  $0.671^{\circ}$  as compared with  $0.661^{\circ}$  for the inner portion, the sap in the more mature portions is probably not diluted to any appreciable extent by sap from the pith and for this reason would not have lower concentrations of sap than more apically placed portions.

From this study of concentration gradients of stem it is evident that when there is a decidedly greater depression of the sap in the terminal portion each succeeding lower portion gives a correspondingly smaller value, so that when plotted in order of distance from the apex these values form a sloping curve. When growth was unusually active in privet the terminal portion gave a very much greater value for the freezing-point depression than the portion next below it. Stems which were not growing actively gave occasionally a greater depression of the freezing-point of the sap of the terminal portion, but the differences in value between different portions were not as great as for more actively growing stems. Also, in all cases when active growth had ceased the sap of leaves gave a greater depression of the freezing-point than the portions of the stem to which they were attached, while leaves from actively growing shoots gave smaller values than the stems. It was shown that the sap of internodes does not uniformly dilute the sap of the nodal region, because it may give either the same value as that of the node above or below, or a greater or smaller value. The pith was found to have practically the same concentration of sap as the wood and phloem.

It appears, therefore, that there is a correlation between the osmotic concentrations of the sap of different portions of stems and the tendency of the buds to develop on those portions. However, due consideration must be given not only to the concentration of the sap of the stems but also to that of the leaves. This concentration of the sap of the leaves is an extremely important factor when the water supply of the plant as a whole is small and when there has been an unusual drain on the water in the tissues in consequence of excessive transpiration.

## DISCUSSION OF RESULTS

The literature on the osmotic relations of plants has been so extensively reviewed in the books of Livingston (19) and Atkins (2) and in the papers of Chandler (8, 9) and Harris and Gortner (15) that discussion here will be limited to the work immediately bearing on the subject of this paper. Although Lutman's (21) method of extraction differed in that he did not freeze the tissue before extracting the sap, it is interesting to note that he always observed a greater concentration of sap in the sprouts than in the tissues of potato tubers. In one case the sprouts showed an increase of  $0.088^{\circ}$  in the freezing-point depression over that of the rest of the tuber. The observed increase of 1.376 percent in glucose and 1.360 percent in sucrose would more than account for this greater osmotic concentration, for such a solution would increase the freezing-point depression by  $0.213^{\circ}$ . However, Butler (7) finds that sugar is not present in all germinating tubers, and he ascribes the distribution of sugar when present in germinating and resting potatoes in part at least to metabolic changes induced by another factor. Both Buckner (6) and Cook (10) record a greater ash content in the sprouts, and Cook has shown a higher nitrogen and phosphoric acid content. Brannon (5) found a greater acidity in the region of the eye and Appleman (1) a greater catalase and respiratory activity in the apical end. All these results are indicative of more rapid metabolic processes in apical portions. Just before growth starts there is an increase in the osmotic concentration of the tuber, which is later followed by a decrease. Therefore the presence of a larger amount of osmotically active substances in a portion of a plant at the critical period would cause a diffusion of water into those tissues. Those portions of the stem may also have an advantage in obtaining the reserve foods because of their higher rate of metabolism.

Butler (7) and Hopkins (17), as well as other observers since Müller-Thurgau (22), have noted changes in the sugars of potatoes along with changes in temperature. That storage conditions may alter varietal tendencies is not strange, for, although Butler confirms the results of Müller-Thurgau that different varieties of potatoes exhibit differences in the rapidity of sugar-accumulation, Cook (10) observed little variation in the chemical composition of different varieties and found that the age of the sprout apparently influences the composition more than the variety. Since in tubers properties other than osmotic concentration may be affected by environmental changes, it is not unlikely that many varietal tendencies now attributed solely to inheritance may be modified by environmental conditions.

Other workers have observed higher concentrations in the sap of leaves or stems near the apex but have not attempted to relate this phenomenon to inhibition (Atkins, 2; Harris and co-workers, 16; Reed, 24). That Reed and others also find a lower concentration in actively growing shoots and a greater concentration in less actively growing ones is an apparent contra-

diction, but an analysis of their work shows that they are considering values from samples composed of the entire shoot together with its leaves. In most of the previous work on stems the samples included tissues from many regions of growth. However, in my own experiments on limiting the amount of tissue to a single, and occasionally to several, nodes, there is a very much greater concentration of sap in the portions toward the apex than in the lower portions of very actively growing shoots (text figs. 10 and 11), while in less actively growing shoots (text figs. 14 and 15) there is only a slightly greater concentration.

When there is active growth (text fig. 11) the average of all the values for the stem and leaf portions is  $1.104^{\circ}$ , and when there is no active growth (text fig. 12) the average is  $1.230^{\circ}$ . These data are comparable with those of Reed and show that composite samples of actively growing shoots may have low concentrations. From this it might be concluded that all parts of less actively growing shoots had a greater concentration of sap than those of more actively growing shoots. However, in general the lower leaves of the less actively growing shoots have a greater concentration of sap than those of more actively growing shoots, and since these leaves have such a large volume of sap this would account for the greater concentration of sap in a composite sample from such tissues.

With unusual conditions of light intensity and moisture content of the air and the soil the osmotic concentrations may show greater irregularities, as indicated in text figure 8. Shading tends to produce a smaller concentration of sap (Chandler, 8; Lutman, 21); and lack of atmospheric moisture may alter the osmotic concentration (Harris, Lawrence, and Gortner, 16).

Conditions in the plants themselves may produce changes in the moisture relations of the different tissues. Pringsheim (23) found in thick-leaved plants, such as *Sedum spectabile*, that young leaves are able to take moisture from older ones. It is demonstrated by the studies of Chandler (9) that fruits may replace in leaves the water lost from those leaves by excessive transpiration. The experiments of Curtis, in which shoots were placed in strong sugar solutions and the lower buds developed most strongly, have already been mentioned.

There is no question but that the relative osmotic concentration of tissues is correlated with the growth of different parts of plants. The substances that produce these differences in concentration may not be the same in all cases, but in the majority of cases sugars and electrolytes, either as minerals or in organic combinations, are the main contributors. Popular opinion associates growth with the presence of nitrogen, and some preliminary experiments indicate that there is a greater amount of total nitrogen per cubic centimeter of sap present in parts having relatively greater osmotic concentrations. Since the nitrogen is in solution, it is probably present in the form of amino acids. There is good evidence that

certain amino acids accelerate not only most metabolic processes (Spoehr and McGee, 27) but also the hydrolysis of starch (Sherman and Walker, 26). Thus, theoretically, with a high nitrogen content there may be produced an increase in solutes and a subsequent increased osmotic concentration.

#### SUMMARY AND CONCLUSIONS

From this study, in which the concentration of the sap of different portions of stems has been determined by the cryoscopic method, the following conclusions were reached:

1. With potato tubers the sap of the sprouts always gave a greater concentration than any portion of the tuber.
2. Tubers in which there was a strong terminal sprout nearly always gave a greater concentration of the sap of the eyes nearest the apex, whereas tubers which sprouted irregularly only rarely showed a slightly greater concentration in the apical eyes than in the basal eyes.
3. Certain varieties of potatoes, having a normal tendency to develop sprouts in a certain fixed manner, not only deviated in their behavior from the normal when the storage conditions varied, or when the degree of maturity varied, or when the season, duration, and conditions of the growing period were different, but also showed changes in the osmotic concentrations of the different portions.
4. Just before the sprouts began to appear there was found an increase in the concentration of the sap of the tubers as a whole, which, with the development of the sprouts, is followed by a decrease due probably to the passage of osmotically active substances into the sprouts or to changes in the substances due to greater respiratory activity associated with growth.
5. The preliminary experiments with *Bryophyllum* stems indicated that the apical buds commonly did not inhibit the lateral and basal buds, and that about as commonly the stems had a smaller concentration of sap in the apical portions than in lower portions.
6. In shoots of privet and *Philadelphus* which did not branch laterally the first year and in which the terminal bud was actively growing, the uppermost portions with very few exceptions were found to have a distinctly greater concentration of sap than those below. In these same shoots the concentration of the sap of the leaves was usually less than that of the stem, and when greater the difference was not significant.
7. On the other hand, in shoots in which the terminal portion had ceased growing actively, although this region usually had a somewhat greater concentration than the lower regions, yet the values for the concentration of the sap of the stems in such shoots did not extend over as wide a range as those growing more actively. Also, the concentration of the sap of the leaves and of different portions varied less.
8. Cessation of active stem-growth was associated with a condition in

which the leaf-sap concentration was relatively high as compared with the sap concentration of the stem.

9. The data on the whole suggest a fairly close correlation between the osmotic concentration of a tissue and its tendency to inhibit growth of other tissues or to be inhibited by them.

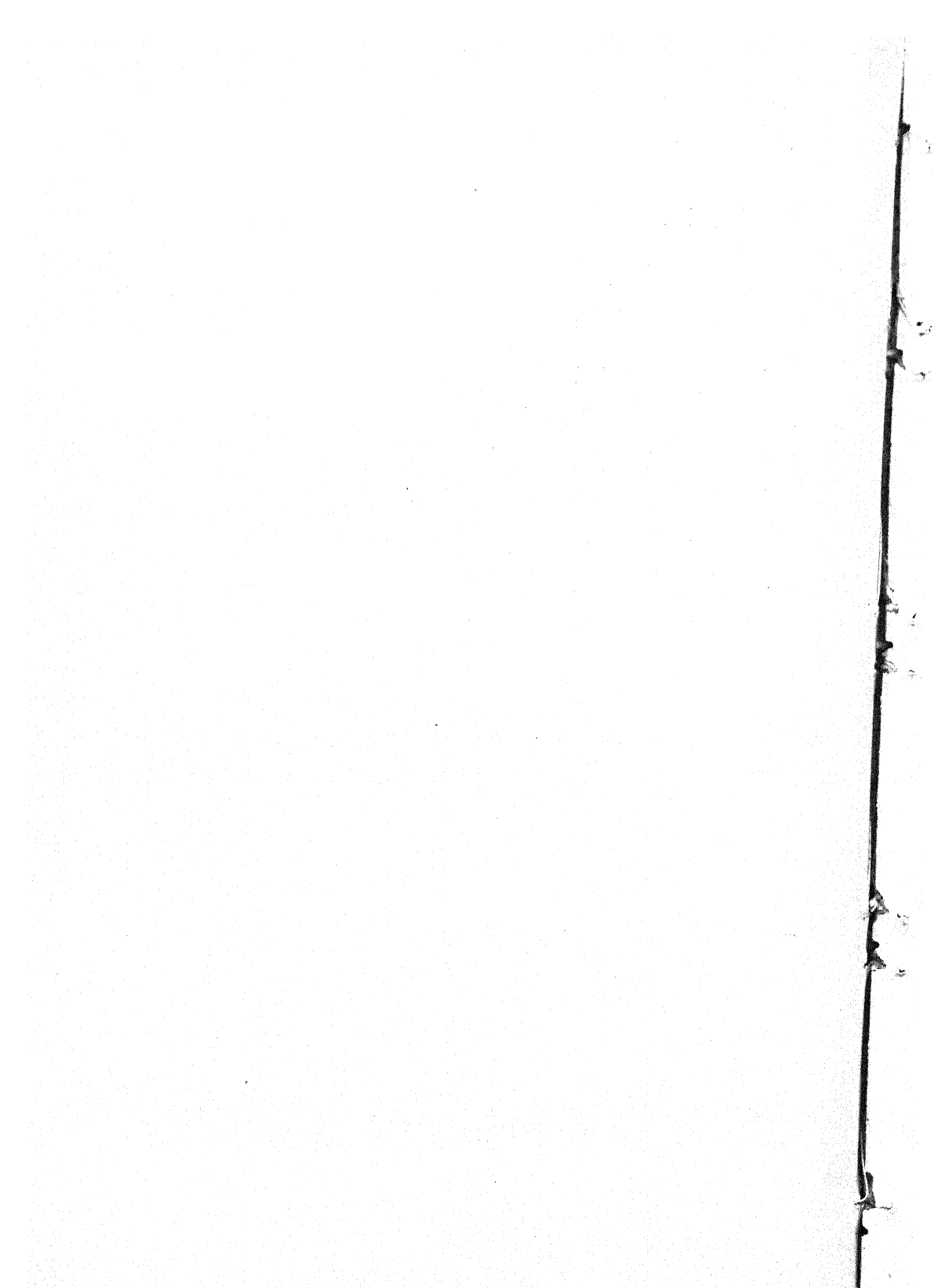
10. While it is evident that osmotic concentration may be an important factor in "inhibition" and may at times be the determining factor and should be considered in experiments on inhibition, it is certainly but one of perhaps several factors.

This investigation was conducted in the laboratory of plant physiology at Cornell University at the suggestion and under the direction of Professor O. F. Curtis. The writer wishes to express to Professor Curtis her appreciation for the advice and encouragement received during the course of the work.

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## SOME OF THE FACTORS INVOLVED IN THE SEXUAL REPRODUCTION OF *MARCHANTIA POLYMORPHA*<sup>1</sup>

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It has rather recently been established by the experiments of Garner and Allard (3) that the relative length of day and night has a decided influence on the time of flowering and the vegetative growth of plants. On the basis of these experiments plants can be rather generally grouped into two large categories, "long-day" and "short-day" plants, depending on the "photoperiod" or length of daily illumination which results in the production of flowers and fruit. This regulatory action of the relative length of day and night has been confirmed by later work of the same authors (4) and by other investigators (2, 9, 12). Oakley and Westover (12) have found in the phenomenon of photoperiodism a means for the quick identification of seed of different alfalfa varieties, and Wanser (15) finds in this regulatory effect the key to the distinctions between winter and spring wheats. The sexual expression of the flax plant has been found by Schaffner (13) to be influenced by the relative length of daylight, and in the case of some animals it has also been demonstrated (7, 10) that sexual reproduction may be affected by the photoperiod.

The explanation of this regulatory effect is as yet purely speculative. The fact that plants can be grown to maturity with normal fruit-production in continuous artificial illumination (5) indicates that altered periodicity of illumination induces changes in the internal cell mechanism of the plant which are responsible for the observed phenomena. Garner and Allard tend to the view that the internal water supply, the degree of hydration, of the living contents of the cell may be the mechanism involved in the plant's

<sup>1</sup> The experiments reported here were started when the writer was an instructor in the department of botany, College of Agriculture, Cornell University, and they are being continued at the same institution under a fellowship in the biological sciences of the National Research Council. To both of these institutions, the writer desires to express his appreciation for facilities placed at his disposal for carrying on the study.

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expression. Others attempt to explain the responses on the basis of an altered carbohydrate-nitrogen ratio (11). In addition to the photoperiod, however, other external environmental factors, such as temperature, humidity, soil conditions, etc., may also play a part in producing the response. Thus temperature alone has been found to exercise a regulatory effect on reproduction in *Zostera marina* (14), and in the present paper it will be shown that humidity may undoubtedly be an important factor in the formation of sexual branches in *Marchantia*.

In most of the investigations cited above, flowering plants have served as experimental material. The plant body, as well as the fruit, in such cases is composed largely of sporophytic tissue. The gametophytic phase, culminating in the sexual fusion of egg and sperm, is also essential to fruit-setting except in rather rare instances. Thus a study of the gametophytic generation, quite apart from the sporophyte, may be important in solving some of the problems of fruit-production. For this reason it seemed desirable to determine the effect of the length of day on a plant like *Marchantia*, in the life history of which the gametophytic stage predominates. A further reason for the investigation of *Marchantia* is because of the rather extensive use of this plant as a representative of the liverworts, or of the bryophytes as a whole, in introductory courses in biology. Ample vegetative material of the gametophytes of *Marchantia* can be secured in winter by growing the plants on cinders in the greenhouse, but for a study of the sexual branches and sporophytes preserved material is necessary, as these structures are produced abundantly in nature only during June and July, though in the greenhouse they occur somewhat earlier. In view of the many recent experiments on periodicity and the effect of artificial illumination on the growth and reproduction of plants, the idea suggested itself that *Marchantia*, being apparently a "long-day" plant, might respond to artificially lengthened days in the winter and thus be brought into proper condition for class study of the sex organs and sporophytes at any desired time.

Accordingly, several cultures of the gametophytes of *Marchantia polymorpha* L. were prepared, using as culture containers ordinary baking tins ( $5\frac{1}{2} \times 10 \times 2\frac{1}{2}$  in.) filled with finely sifted cinders. A constant and fairly uniform water supply was furnished each culture by means of two cylindrical atmometers which were buried just beneath the surface of the cinders and connected by a siphon to a small external water reservoir. In the first series, started in September, 1922, four such containers were planted with gemmae and pieces of the mature gametophytes of *Marchantia*. At the end of a month, during which all the cultures were exposed to similar environmental conditions in the greenhouse, the plants were fully established and growing vigorously. They were then subjected to various daily photoperiods.

For securing a daily exposure to light shorter than the normal day, a

dark box was used (Pl. XXVIII, fig. 1). This box, divided by partitions into three compartments, was covered with several layers of black paper, ample provision being made for ventilation without the admission of light. The culture containers and water reservoirs were mounted on small cars which operated on tracks extending from the dark-box compartments out on to the platform in front of the box. By moving the cars into and out of the box the daily exposure of the plants could thus be readily controlled. The operation of placing the cultures in the dark box was accomplished automatically by attaching a suspended weight to the rear of each car, so that, when released from its position on the platform, each car would be drawn into the box by the falling weight. During the daily exposure of the cultures the cars were held in position on the platform by means of spring catches controlled by alarm clocks. Each car on entering the dark box also closed the compartment automatically by tripping the wire support of the sliding door. The apparatus thus required attention only once during the 24-hour period (at 8 A.M.) when all the cultures were placed in position for exposure to light. The length of the daily exposure could readily be altered either by changing the time set on the alarm clocks or by transferring the culture containers from one car to another. In order to overcome any slight differences in the environmental conditions of the various cultures, the latter procedure was adopted. A check culture remained in a stationary position on the platform.

Beginning October 6, 1922, the four cultures received daily exposures to light of 6, 8, 10 hours, and all day (check). On November 27 a fifth culture was added to the series, this culture receiving 6 hours' artificial light at night from a single 100-watt mazda lamp in addition to the normal daylight. This culture, which will be referred to as the "long-day" culture, was also mounted on a car similar to those described above, but operating on a track which extended along the outside of the dark box. The car, when released from its position on the platform, was drawn down the track, automatically turning on the electric light which illuminated the culture till midnight. The plants in this first "long-day" culture were obtained from the four other cultures by transplanting.

The results of the first experiment are presented in table 1. From October 6, 1922, until February 1, 1923, cultures 1 to 4, receiving daily exposures of 6, 8, 10 hours, and normal daylight respectively, remained sterile. Culture 5 received a "long day" starting November 27, 1922, and on December 11 the first indication of sexual branches was observed. By the end of January, 1923, these branches were mature.

On February 1, 1923, the cultures were shifted to new positions on the apparatus, resulting in increased daily exposures for nos. 1, 2, and 3, and in shorter exposures for nos. 4 and 5. Culture no. 3 responded at once to the "long-day" exposure, producing mature antheridiophores in 20 days. Many archegoniophores were mature on this culture early in March, and

TABLE 1. *Effect of the Length of Day on Sexual Reproduction in Marchantia polymorpha L.*<sup>2</sup>

Date	Culture No. 1		Culture No. 2		Culture No. 3		Culture No. 4		Culture No. 5	
	Daily Exp.	Condition	Daily Exp.	Condition	Daily Exp.	Condition	Daily Exp.	Condition	Daily Exp.	Condition
Oct. 6, 1922	8 A.M. to 2 P.M.	Sterile	8 A.M. to 4 P.M.	Sterile	8 A.M. to 6 P.M.	Sterile	All day	Sterile	Nov. 27, long day	Sterile. Dec. 11, first sex org. Dec. 23, most of branches sexual.
Feb. 1, 1923	8 A.M. to 4 P.M.	Sterile	8 A.M. to 6 P.M.	Sterile	Long day	Sterile Feb. 20, mature anth., young arch.	8 A.M. to 2 P.M.	Sterile	All day	Mature sex org. Good veget. growth.
Mar. 1, 1923	8 A.M. to 4 P.M.	Good veget. growth. 3 young anth., no arch.	8 A.M. to 6 P.M.	Good veget. growth. A few anth., no arch.	Long day	Mar. 19, mature arch. Transf. sperms	All day	10-12 anth., 20-25 arch.	Long day	New sex organs start. Many new arch.
May 1, 1923	8 A.M. to 3 P.M.	Sterile June 5, sterile	All day	6-8 mature anth., many young arch.	All day	Spores shed. Good veget. growth. New arch.	All day	All stages. Many mature arch.	All day	Good veget. growth. Old arch. dead.
Aug. 15, 1923	8 A.M. to 6 P.M.	Sterile. Sept. 29, good vegetative growth. Produced 3 or 4 anth. during the summer.								
Nov. 1, 1923	Long day	Sterile Nov. 8, first antheridiophores. Dec. 3, anth. mature, many arch. half grown; sperms transf. Dec. 30, spores.								

<sup>2</sup> The following abbreviations are used in the tables: anth. = antheridiophore(s); arch. = archegoniophore(s); veget. = vegetative; sex org. = sexual branches; transf. = transferred; exp. = exposure. Under "Daily Exp.," "All day" means exposure to the full normal daylight for that period; "Long day" means exposure to the full normal daylight plus artificial light at night.

on March 19 artificial transfer of sperms was accomplished by placing large drops of water on the expanded tops of the antheridiophores and, after a moment, transferring portions of the drops with the contained sperms to the tips of the younger archegoniophores. Sporophytes were produced, and were shedding spores on May 1. The condition of this culture on March 19 is shown in Plate XXVIII, figure 2.

Further changes in the exposures of the cultures were made on March 1 and May 1, with the results indicated in the table. The case of culture 4 is of interest. Sexual branches were produced on this culture under the normal-day exposures of March after a previous month of 6-hour-day exposures, indicating that the actual relative length of day to night may not, in itself, be the determining factor in "fruiting." Internal changes caused by the sudden radical change in the daily amount of light received by the plants are probably responsible. The same situation occurred in the second series of cultures, as will be pointed out later.

Culture 1 was kept in a sterile condition for over a year (with the exception of the production of 3 or 4 antheridiophores during the early summer) by maintaining it in daily exposures of 10 hours or less. On November 1, 1923, this culture was changed to the "long-day" exposure. Within a week the first evidences of sexual branches were observed, and by the end of the month several antheridiophores were mature and many young archegoniophores were present. Artificial transfer of sperms was accomplished on December 3. Sporophytes resulting from the fertilizations were mature and shedding spores by the end of December. (See Pl. XXIX, figs. 1 and 2, for condition of this culture on October 29 and December 17, 1923.)

On May 1, 1923, a new series of cultures was installed on the apparatus with the idea of repeating the first experiment during the longer days of spring and summer. In this series a considerable amount of garden soil was mixed with the cinders in the culture containers. The *Marchantia* plants were grown entirely from gemmae which were selected from stock cultures of both male and female gametophytes. The results of the test are presented in table 2. The response of the plants to a "long day" was again very evident. Sexual branches began to appear on culture 4 (the "long-day" culture) almost at once. In consequence of clogging of the irrigation system, however, the culture dried out somewhat, with the result that only five of the archegoniophores matured.

Following a change in the positions of the cultures on August 15, culture 2 responded immediately to the "long-day" exposures, maturing many sexual branches. Transfers of sperms were made on September 15. Sporophytes were produced in abundance, and many of these were shedding spores by the middle of October. The condition of this culture on October 29 is shown in the photograph, Plate XXX, figure 1.

Culture 3, the check, remained sterile all summer, but responded later

TABLE 2. *Effect of the Length of Day on Sexual Reproduction in Marchantia polymorpha L.*

Date	Culture No. 1		Culture No. 2		Culture No. 3		Culture No. 4	
	Daily Exp.	Condition	Daily Exp.	Condition	Daily Exp.	Condition	Daily Exp.	Condition
May 1	8 A.M. to 3 P.M.	Sterile	8 A.M. to 6 P.M.	Sterile	All day	Sterile	Long day	Sterile. May 20, one young arch. June 4, 5 arch.
Aug. 15	All day	Sterile Aug. 27, young sex org.	Long day	Sterile. Aug. 18, tips turn up. Aug. 25, sex org. Sept. 15, mature sex org. Sperms transf.	All day	Sterile Produced two anther- idiophores during the summer.		
Sept. 26	Long day	New sex org. ap- pear.	All day	Young sporophytes.	All day	Sterile		
Oct. 6	All day	Nov. 5, many arch. and anth. All stages.	All day	Oct. 25, spores shed. Nov. 5, many mature sporophytes.	Long day	Sterile Nov. 5, sexual branches starting on nearly all growing tips.		

to "long-day" exposures. The failure of this culture to "fruit" during June and July, even though exposed to the full normal daylight, may possibly be attributed to the soil-cinder mixture used as a culture medium in this experiment. A much better vegetative growth of the plants was obtained on the pure cinders in experiment 1 than on the soil-cinder mixture of experiment 2.

Culture 1 started to form sexual branches under the normal-day exposures of August after previous daily exposures of 7 hours from May 1 to August 15, again indicating that a sudden radical change in the photo-period may result in the production of sexual branches. On September 26 this culture was changed to the "long-day" exposure, and many new sexual branches soon began to appear.

Culture 3 remained sterile all summer (except for the formation of two antheridiophores) and on October 6 was changed to the "long-day" exposure. By the end of the month sexual branches were beginning to be formed on nearly all the plants.

On November 5 practically all stages in the life history of *Marchantia* were represented by the three cultures, 1, 2, and 3. Vegetative plants and very young sexual branches were present on culture 3; mature antheridiophores and nearly all stages in the development of the archegoniophores were found on culture 1; sporophytes in various stages up to those shedding spores were present on culture 2.

Results of a similar nature were obtained on a somewhat larger scale with a bed of *Marchantia* on one of the greenhouse benches. This bed, containing fine cinders, was planted with pieces of *Marchantia* in the fall of 1922, and by March 1, 1923, it was covered with vegetative plants. There was no evidence of "fruiting" on any of the thalli. Beginning on March 1 one half of the bed received artificial light at night in addition to the normal daylight, the other half receiving only the normal daylight. Plants on the illuminated or "long-day" side responded at once to the additional light. Young antheridiophores were observed on March 16, and by March 21 young archegoniophores were in evidence. Many of the latter were mature on April 1, and by May 1 many of the plants contained shedding sporophytes. The photographs (Pl. XXX, fig. 2, and Pl. XXXI, fig. 1) show the conditions of the two portions of the bed on April 30. About the middle of May young archegoniophores began to appear on the "short-day" side of the bed, though most of the plants were sterile but vigorously vegetative and with abundant gemmae.

A third series of cultures on the small irrigated containers was started August 30, 1923. Gemmae from a single male thallus were sown on each of the containers, in addition to mature pieces of a single male and a single female plant. On November 21, two of these cultures were placed in a compartment of a large glass case, the atmosphere of which was maintained practically saturated with water vapor. The remaining cultures were

TABLE 3. *Effect of Humidity and the Photoperiod on Sexual Reproduction in Marchantia polymorpha L.*

Sexual reproduction in <i>Marchantia polymorpha</i> L.									
Date	Moist Chamber				Dry Chamber				
	Culture No. 1			Culture No. 2		Culture No. 3			Culture No. 4
	Daily Exp.	Condition		Daily Exp.	Condition	Daily Exp.	Condition	Daily Exp.	Condition
Nov. 21	All day	Sterile		All day	Sterile	All day	Sterile	All day	Sterile
Dec. 3	Long day	Sterile Dec. 14, first anth. Dec. 19, many anth., several arch. Dec. 24, mature anth., large arch.		All day	Sterile Dec. 22, young anth. No arch.	Long day	Sterile Dec. 15, first anth. Dec. 22, many young anth. No arch.	All day	Sterile
									Sterile



placed in a dry compartment of the same case. On December 3 an electric light was arranged outside the case in such a manner that one culture in each compartment could be illuminated by it. A single 60-watt mazda lamp was used, the light being turned on at 7 P.M. and remaining on all night. All four cultures thus received the normal daylight, but one culture in each compartment received in addition about 13 hours' artificial illumination.

The results of the experiment are presented in table 3. The vegetative growth of the plants in the moist chamber was markedly stimulated, though the thalli were thin and delicate. In the dry chamber very little extension of the vegetative branches was noted. The "long-day" plants in both chambers soon showed evidences of sexual branches. By December 26 many mature antheridiophores and several well developed archegoniophores were present on culture 1 (long day, moist chamber). Practically all the thalli developed from the male gemmae planted on this culture produced normal antheridiophores. On culture 3 (long day, dry chamber) no archegoniophores were observed, but nearly all the plants from the male gemmae produced antheridiophores which were small and on very short stalks.

Of the normal-day cultures, the one in the dry chamber remained sterile; in the moist chamber, however, antheridiophores were produced on the young male plants, even though they received only the normal daylight of mid-December. No archegoniophores were observed on this culture.

Any explanation of the results obtained with this last series of cultures must be purely speculative until data are available on the internal changes occurring in the plants. If the internal conditions in cultures 2 and 3 were somewhat similar as regards total carbohydrates and nitrogen, it must be assumed that there was a considerable difference in the rates at which carbohydrates accumulated in the two cases, since the nitrogen content of the medium was essentially the same in all four cultures of this series. An attempt to explain the response on the basis of available water supply also meets with difficulties. Thus Kraus and Kraybill (8) found in the case of the tomato that abundant moisture and minerals, including nitrates, without available carbohydrates resulted in weakly vegetative and non-fruitful plants, whereas with an available carbohydrate supply increased vegetation and sterility resulted. It might be argued that these conditions existed in cultures 2 and 3 respectively. Both, however, produced antheridiophores.

Since archegoniophores were not produced in either of these cultures, there is also the possibility that the male gametophyte may be able to produce sexual branches under a greater range of external conditions than the female. In practically all cases where "fruiting" occurred in the mixed cultures the antheridiophores appeared considerably earlier than the archegoniophores. The difference in sex may therefore be practically physiological rather than somatic. The solution must, however, await further evidence.

The results of the cinder cultures in general seem to indicate that *Marchantia* is a "long-day" plant and that by proper control of the length of daily exposures to light large numbers of *Marchantia* plants can be brought into the "fruiting" condition at any desired time. All stages in the life history may then be presented for class study in the living condition. By growing the plants in containers with subirrigation, it is possible to make transfers of the sperms to the archegonia artificially, and the motile sperms themselves can be easily secured for examination. This method of culture might also be advantageous to those interested in studying the early stages in the development of the sexual branches, as it is evident that these must be initiated soon after transferring plants from "short-" to "long-day" exposures to light.

In addition to the mixed cultures on cinders, pure cultures of the male and female gametophytes of *Marchantia* were grown on a variety of mineral nutrient agar media, and some evidence was obtained indicating a possible relation between the nitrogen content of the medium and sexual reproduction. In several instances antheridiophores and archegoniophores were produced on media in which the nitrogen supply was reduced considerably below that of the normal nutrient solution. Beyerinck's solution was used as a standard, with the substitution of  $\text{Ca}(\text{NO}_3)_2$  as a nitrogen source in place of  $\text{NH}_4\text{NO}_3$ . One percent glucose was supplied. The medium of the "fruiting" cultures shown in the photographs (Pl. XXXI, figs. 2, 3) contained one eighth the normal amount of nitrogen. Antheridiophores were also produced on a medium containing one half the normal nitrogen supply but with 2 percent glucose and an exposure to "long-day" conditions. Gametophytes grown on media containing larger amounts of glucose, or with a more readily available water supply, remained sterile.

#### GENERAL CONCLUSIONS

*Marchantia polymorpha* gametophytes respond to the photoperiod in a manner similar to that characterizing "long-day" plants among the flowering plants.

When subjected to artificially lengthened days in the winter, mature antheridiophores are produced in 3 to 4 weeks, mature archegoniophores in 6 to 8 weeks, and mature sporophytes in 10 to 12 weeks. It is thus possible to bring plants into proper condition for class study at any desired time by controlling the photoperiod.

A relatively high humidity tends to hasten the sexual response; a relatively low humidity tends to retard or may inhibit the production of sexual branches, especially archegoniophores.

A relatively low nitrogen-high carbohydrate ratio in the culture medium may result in the formation of sexual branches.

No attempt was made in these experiments to control the temperature.

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## EXPLANATION OF PLATES

## PLATE XXVIII

FIG. 1. The apparatus used in obtaining the various daily exposures of the *Marchantia* cultures to light. The culture nearest the observer is the check or "all-day" culture. The next two cultures receive short-day exposures. One of these is shown entering the dark box. The last is a "long-day" culture and is fruiting abundantly.

FIG. 2. Culture 3 of the first experiment. This culture received "long-day" exposures beginning February 1. Photographed March 19.

## PLATE XXIX

FIG. 1. Culture 1 of the first experiment photographed October 29, 1923. This culture remained sterile for over a year under daily exposures to light of 10 hours or less.

FIG. 2. Culture 1 of the first experiment, photographed December 17, 1923. Many sexual branches have matured during six weeks of daily exposures to "long-day" conditions.

## PLATE XXX

FIG. 1. Culture 2 of the second experiment, photographed October 29. This culture received "long-day" exposures to light beginning August 15. Sexual branches were mature by mid-September, when sperm transfers were made. By the middle of October sporophytes were mature and shedding spores.

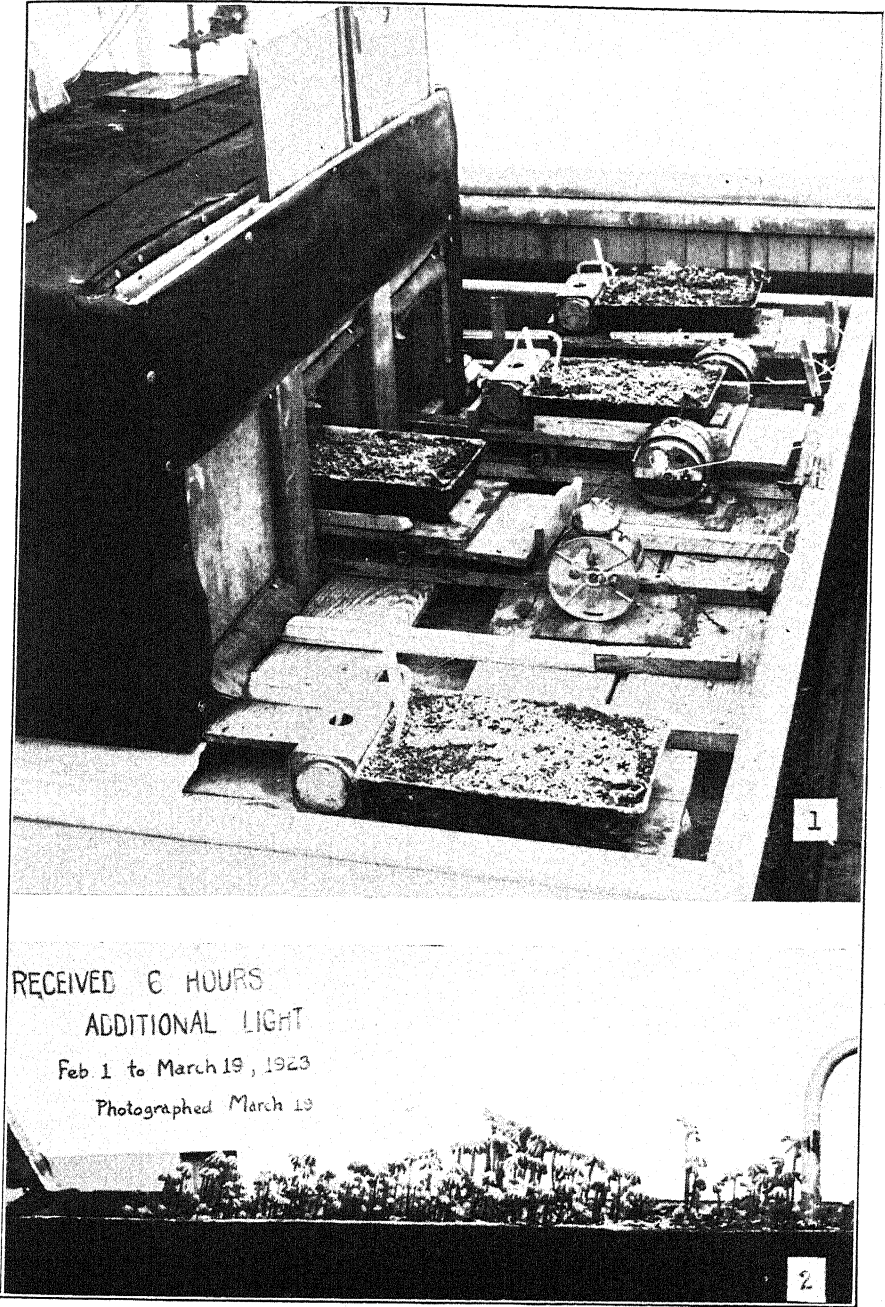
FIG. 2. A portion of the bed of *Marchantia* which received normal daylight only. All the plants are sterile. Photographed April 30.

PLATE XXXI

FIG. 1. A portion of the bed of *Marchantia* which received artificial illumination at night, in addition to the normal daylight, beginning March 1. Photographed April 30.

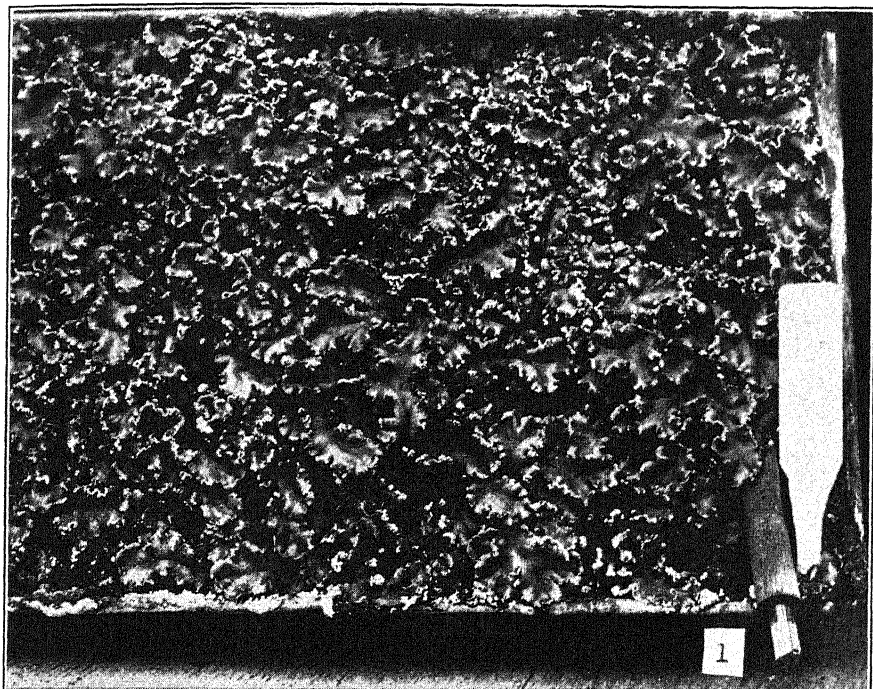
FIG. 2. Pure culture of the male gametophyte of *Marchantia* on mineral nutrient agar containing 1 percent glucose. The nitrogen content of the medium was reduced to one eighth that of the normal nutrient solution.

FIG. 3. Pure culture of the female gametophyte of *Marchantia*. The medium is the same as that of the culture shown in figure 2.



WANN: SEXUAL REPRODUCTION OF MARCHANTIA

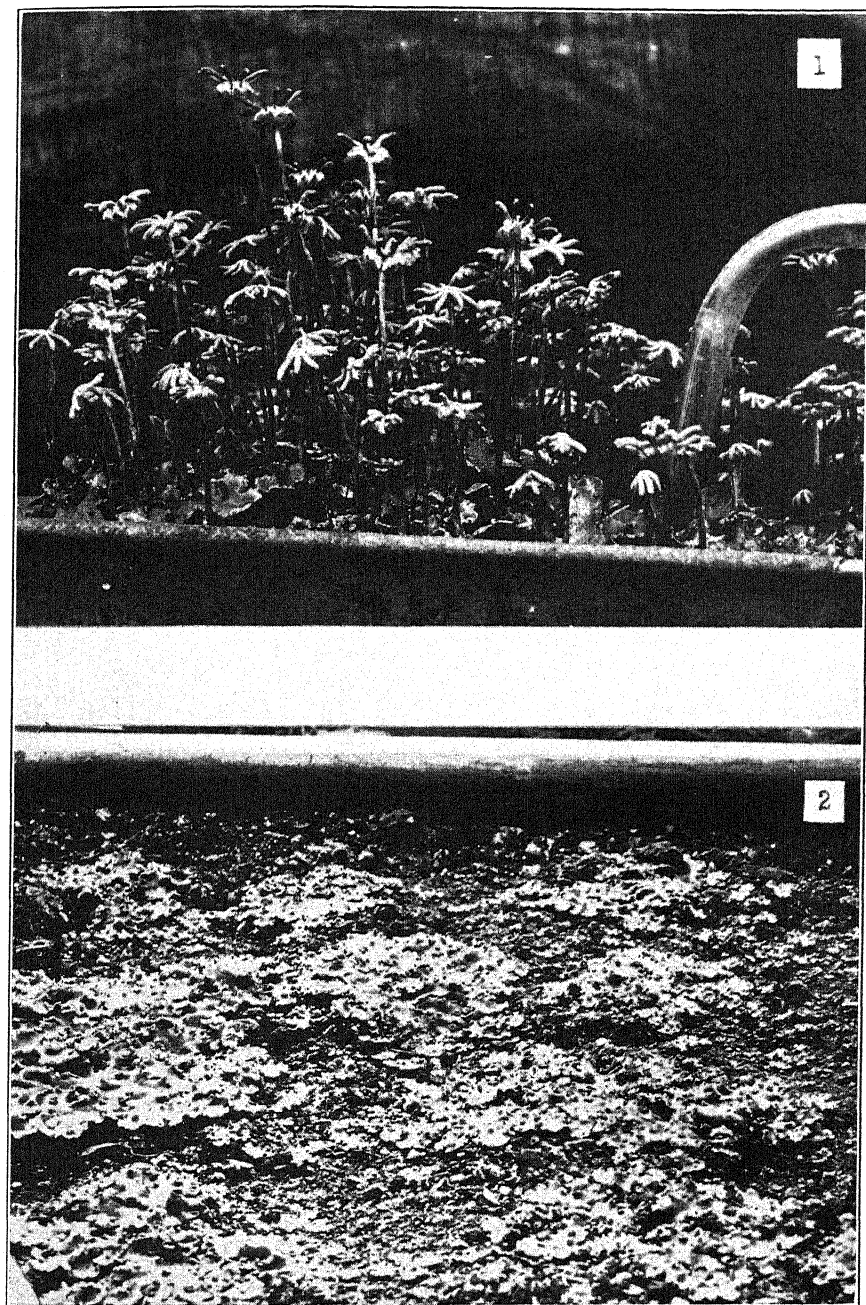




WANN: SEXUAL REPRODUCTION OF MARCHANTIA

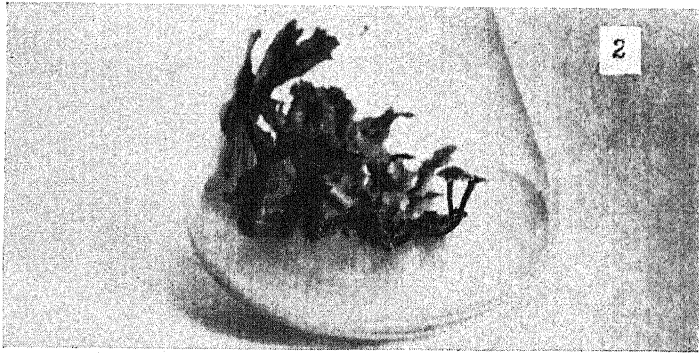
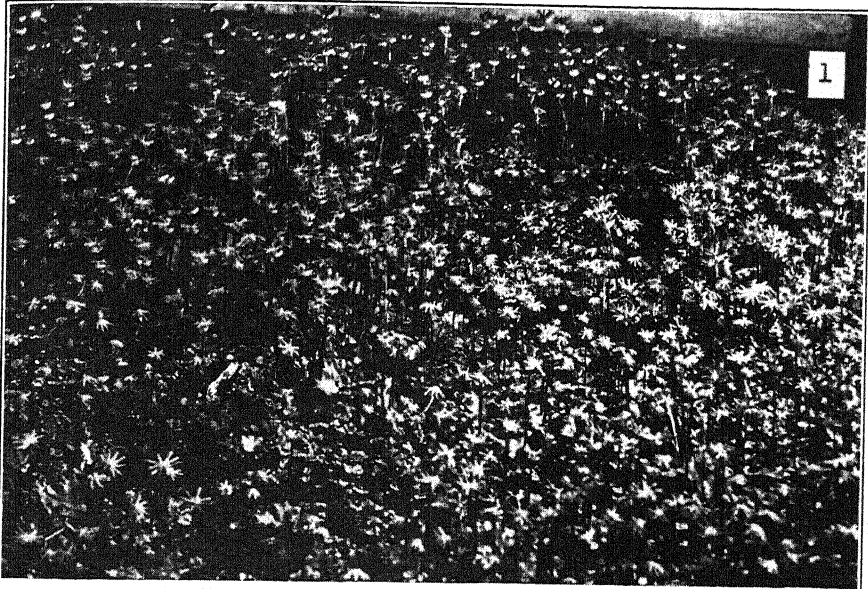






WANN: SEXUAL REPRODUCTION OF MARCHANTIA





WANN: SEXUAL REPRODUCTION OF MARCHANTIA



## STUDIES ON ABERRANT FORMS OF *SPHAEROCARPOS* *DONNELLII*

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The studies here reported were made on two aberrant forms of *Sphaerocarpos Donnellii* Aust. which were first observed by Dr. C. E. Allen in his cultures which have been growing under greenhouse conditions since early in 1916. Clones may be kept growing indefinitely under the conditions described by Allen (1919), and it is from clones of races showing the respective characters that the following descriptions are drawn.

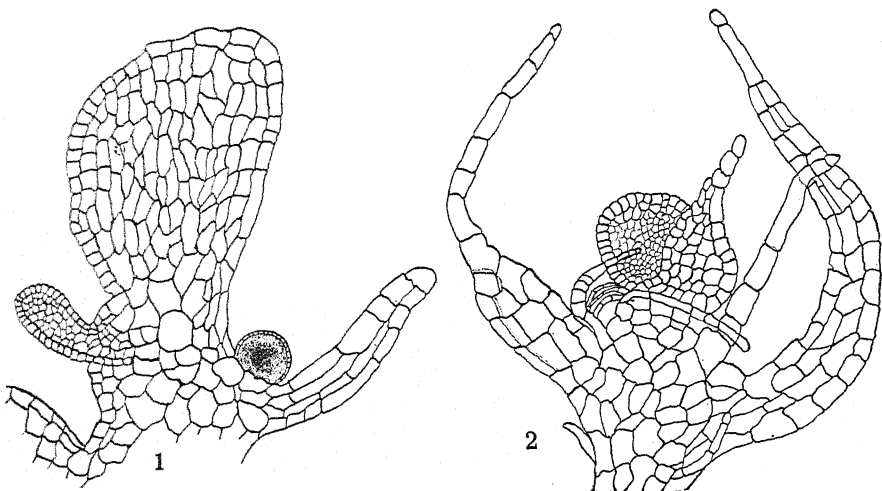
### THE POLYCLADOUS FORM

Two aberrant male plants, probably having a common origin, were isolated from a mixed culture of *S. Donnellii* and used as the starting-points of two clones. To these clones the term "polycladous" was applied.

The most striking character exhibited by the polycladous males is the usual absence, or great reduction, of antheridial involucre. The exposed antheridia in consequence appear under the hand lens or binocular microscope as small green (when immature) or white (when mature) globules borne upon the thickened central portion of the thallus (text fig. 1). Polycladous males produce fewer antheridia than typical males. External factors play an important rôle in determining the number of antheridia on plants of this form, but when the typical and polycladous plants are grown under the same conditions there is always a larger proportion of antheridia produced on the former. Indeed, under some conditions, large parts of a polycladous culture may be entirely sterile—a condition never found in the typical form. Figure 1, Plate XXXII, is a photograph of a typical male culture showing numerous antheridia (each enclosed by its involucre). Figure 5 of the same plate shows the general appearance of a polycladous male culture. It may be concluded that this relative infertility of the polycladous form is a fundamental characteristic. The naked antheridia are frequently subtended by short spatulate lobes one cell in thickness (text fig. 1). These structures are also seen on the polycladous male progeny<sup>1</sup> of crosses between the typical female and the polycladous male; and in addition some rare antheridia are surrounded by involucre of approximately the typical form, and some are partially surrounded by variously shaped lobes that seem to be intermediate between typical

<sup>1</sup> The  $f_1$  progeny of a cross between a typical female and a polycladous male includes polycladous and typical females and polycladous and typical males.

involucres and the spatulate lobes. These structures suggest that the spatulate lobes may be reduced involucres. Some of the involucres of the males of hybrid origin have a wide funnel-shaped opening at the top. There is no evidence that this type of involucre is an intermediate form.



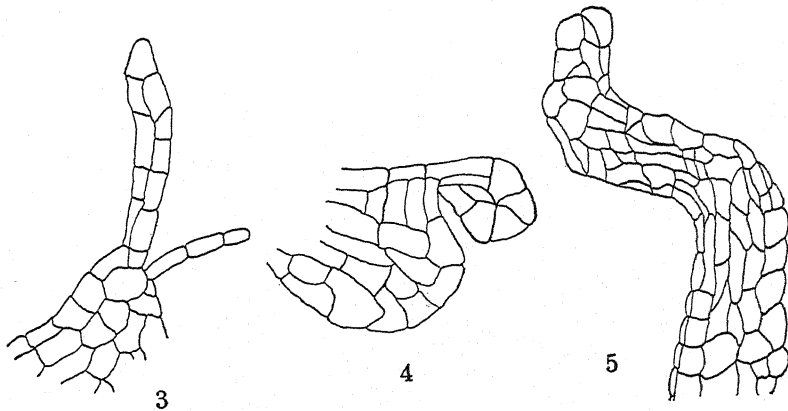
TEXT FIG. 1. Thickened central portion of the thallus of a polycladous male bearing lobes and, at the right, a naked antheridium subtended by a short spatulate lobe. TEXT FIG. 2. Portion of a polycladous male thallus bearing hairs and lobes of varying length.

Another characteristic of this polycladous form is the irregularity of the lateral lobes of the thallus, which are frequently cut or divided in a very variable way. They are, so far as has been observed, always one cell in thickness, but the divisions of the lobe may vary from filaments of single cells placed end to end to flat plates ten or more cells wide. The outlines of the lateral lobes are thus much more uneven than is the case with the lobes of a typical plant. There are also numerous dorsal lobes similar to the lateral lobes just described, and the total number of lobes is much larger than in the case of the typical plant. Figures 2 and 3, Plate XXXII, the former a photograph of a polycladous female, the latter of a typical female, culture, illustrate something of this difference. Branching is frequent and irregular, which fact, together with the irregularly much-lobed appearance of the plant, suggested the name "polycladous." Cilia, each consisting of a single row of cells, are frequently observed as outgrowths of the margins of the lobes (text fig. 3). Similar cilia, variable in length, occur in great abundance on the dorsal surface of the thickened part of the thallus (text fig. 2).

The general appearance of the culture suggests a plant with upright, leafy shoots, but whose leaves (lobes) vary between wide extremes of size and shape. The polycladous males of hybrid origin exhibit the same di-

vergent characteristics as the original polycladous form. They do not seem to be intermediate between the typical and the polycladous form, though in a few clones there are somewhat more involucre forms suggesting a transition to the typical shape than are seen in the original polycladous races. Since these polycladous males of hybrid origin, as well as the original polycladous males, exhibit partial sterility (in the sense of producing few, and at times no, antheridia), it may be concluded that this characteristic is hereditary.

Another notable characteristic of the polycladous males is the occasional presence of a mass of cells imbedded in the thickened portion of the thallus. These masses are entirely internal and are not visible in the living plant. They are irregular in shape without a definite jacket layer. Their form is apparently determined by the position of the surrounding vegetative cells of the thallus. The cells constituting such masses have not proved satisfactory for study, either because of poor fixation or because they were in a degenerating condition when fixed. However, it has been observed that they are small, cubical cells arranged in rather regular rows like the spermatogenous cells of an antheridium. Their appearance suggests that these imbedded masses of cells may possibly represent internal antheridia.



TEXT FIG. 3. Margin of a lobe of a polycladous female bearing hairs. TEXT FIG. 4. Margin of a lobe of a polycladous female with a lobe-like outgrowth. TEXT FIG. 5. Columnar upgrowth from the central portion of a polycladous female thallus.

The polycladous females which appeared among the progeny of polycladous males mated with typical females are functionally sterile. One such female clone (21.56) has been found to produce a few archegonia borne in involucre forms of approximately typical form or subtended by reduced lobe-like structures. Very rarely approximately typical involucre forms have appeared in other polycladous female clones, but no archegonia have been seen except in clone 21.56. The hairs and other peculiarities, including

dorsal lobes, which characterize the polycladous males are found also on the polycladous females, including those of the clone which produced a few archegonia. There are also peculiar upgrowths from the thickened part of the thallus (text fig. 5). These are very variable in size and shape. Each consists of a solid mass of green tissue in the form of a straight or curved rod of approximately the same length as typical involucre. Some taper somewhat toward the tip, like that shown in text figure 5; others are of the same thickness throughout their length; still others are broader at the tip than at the base, the tip being sometimes concave (vase-like); and some fork toward the apical region. The position of these upgrowths on the thallus leads to the conclusion that they may be distorted involucres. The general appearance of the polycladous females is similar to that of the polycladous males except in size and in the fact that they frequently bear these columnar upgrowths. The polycladous forms display the same characteristic differences in size between male and female as does the typical *S. Donnellii*.

#### THE SEMI-STERILE FORM

The "semi-sterile" character is as yet known only in males. Numerous clones showing this character, all probably having a common origin, were isolated from one mixed culture.

In so far as has been observed, the semi-sterile males differ from the typical males in only one respect, that of the proportion of antheridia and antheridial involucres produced. They have been called "semi-sterile" because a plant of one of these races rarely produces as many antheridia as does a typical male plant. An occasional branch is fully fertile; quite frequently some branches are entirely sterile; sometimes a whole culture is sterile—that is, no antheridia or involucres are visible (Pl. XXXII, fig. 4). However, when it does produce a fairly large number of antheridia, a semi-sterile male plant is not visibly different from a typical male. *Sphaerocarpos*, and indeed the *Hepaticae* in general, are extremely responsive to changes in the environment. The fact, stated in regard to the polycladous form, that external factors play an important rôle in determining the number of antheridia, is true also of the semi-sterile males and to some extent of the typical males. But it is also true that when the semi-sterile male is grown in the same environment as the typical male there are always more antheridia produced on the latter; and, as has been stated above, the semi-sterile males are frequently and for long periods of time entirely sterile, whereas the typical males are never so.

#### THE CHROMOSOMES OF THE ABERRANT FORMS

##### Polycladous Females

In the gametophytes of two polycladous female clones, 21.56 and 20.366, chromosome figures were seen in cells of the apical meristem. The



metaphases, especially when seen in polar view, are best for the study of the chromosomes, as they are more spread out during these stages and so are more easily counted and examined. The chromosomes exhibit a marked tendency to lie close together. This crowding may be due to fixation or to subsequent treatment, but this is not likely as the crowding is noticeable in all the preparations.

The chromosome number in each dividing nucleus of the polycladous female is eight (Pl. XXXIII, figs. 1-5). Seven of the chromosomes vary in length among themselves and have the form of slender, irregularly curved rods. They can not be identified with certainty in the various figures. The determination of the length of a chromosome is a very difficult task, since in the preparations all parts of a chromosome do not lie in the same plane. As pointed out by Allen (1919), the study of a large number of figures is necessary for such identification. The thickness of these seven chromosomes is quite uniform, and does not vary to any great extent even as between different cells or different plants. The X-chromosome (Allen, 1917, 1919) is always longer and thicker than any of the others. It seems to vary in length and thickness in different cells, but is always the largest and most conspicuous. This chromosome, possibly because of its size, seems to be more sluggish than the others in its movements during mitosis; its daughter halves are the last to separate in the metaphases (Pl. XXXIII, fig. 5). This peculiarity has been noted by Allen (1919) in typical *S. Donnellii*, and by Miss Schacke (1919) in *S. texanus*.

The culture described above as a polycladous female (20.366) is so considered because of its size and vigor, and because the chromosome condition in it is the same as in that of the other clone studied, which produced a few archegonia.

The points of attachment of the spindle fibers, determined by the shapes of the chromosomes as they pass to the poles of the spindle, are seen to vary among the different chromosomes (Pl. XXXIII, fig. 5). In some cases the fibers seem to be attached at the ends of the chromosomes, causing the latter to assume the form of straight rods; in other cases the fibers are attached near the center, so that some chromosomes are U- or V-shaped. A comparison of a considerable number of spindle figures as to points of attachment of the fibers might render valuable aid in identifying the chromosomes. In Plate XXXIII, figure 5, the spindle fibers are apparently attached near the end of the X-chromosome, but Allen (1919) figures this chromosome in the form of a U. Miss Schacke also finds it to be U-shaped. The results so far obtained do not warrant any conclusion upon this point.

### Polycladous Males

Chromosome figures were seen in the vegetative meristem and in the spermatogenous cells of antheridia of the polycladous male clone 20.374.

Here again the metaphases are the most favorable for the study of the chromosomes. Each dividing nucleus in the male also shows eight chromosomes (Pl. XXXIII, figs. 6-9). Seven of these are similar to the seven smaller chromosomes of the female gametophyte, but on account of the difficulties mentioned above they have not been identified individually. The eighth chromosome is very much smaller than any of the others and corresponds to the Y-chromosome of the typical male (Allen, 1919). This chromosome appears spherical or sometimes very slightly elongated.

### Semi-sterile Males

The dividing nuclei in the semi-sterile male R27B also show eight chromosomes, one of which is very much smaller than the others and is evidently the Y-chromosome. The chromosome group of a semi-sterile male (Pl. XXXIII, figs. 12, 13) is not visibly different from that of a typical male (figs. 10, 11) or from that of a polycladous male (figs. 6-9).

### DISCUSSION

The large chromosome observed in the polycladous females, and described above, is doubtless to be identified with the X-chromosome of *S. Donnellii* and with the corresponding body of *S. texanus*. The chromosome condition in the aberrant polycladous females is thus similar to that in typical *S. Donnellii* and in *S. texanus*. The small chromosome of the polycladous males and that of the semi-sterile males may similarly be identified with the Y-chromosomes of typical *S. Donnellii* and of *S. texanus*.

These results do not furnish evidence upon which conclusions as to the origin of the polycladous and semi-sterile forms might be based. They present merely negative evidence that the aberrant forms were not produced by non-disjunction of the chromosomes, and do not preclude the possibility of mutation. They offer some evidence that these forms are variants of *Sphaerocarpos* and not accidental intrusions of distinct forms—as might be suspected from the atypical appearance of the polycladous forms especially. This conclusion is supported by the fact that both the polycladous and the semi-sterile males cross readily with the typical females—a fact that is rendered more striking by the complete infertility thus far of attempted matings between *S. Donnellii* and the very similar *S. texanus*.

### SUMMARY

1. Polycladous male plants are characterized by the absence or aberrant development of antheridial involucre, scarcity of antheridia, irregularity in branching, varied form of thallus lobes, and the presence of cilia. Polycladous females are characterized by the almost complete absence of archegonia, complete functional sterility, and the presence of dorsal columnar upgrowths, as well as irregularity in branching and in lobe form, and the presence of cilia.

2. Semi-sterile males differ visibly from the typical males only in the much less numerous antheridia and antheridial involucre.

3. The chromosome number in the cells of polycladous females, offspring of typical *Sphaerocarpos Donnellii* (female) crossed with a polycladous male, is eight, as is also the chromosome number of the polycladous male gametophytes of similar origin.

4. In the polycladous females one large chromosome is always present; in the polycladous males this body is never present, but one small chromosome appears in its stead.

5. The chromosome number in the semi-sterile males is likewise eight, including a small element similar to that observed in polycladous males.

6. The chromosome condition in the polycladous males and females and in the semi-sterile males is similar to that in the corresponding sexes of typical *S. Donnellii* and *S. texanus*, indicating that the aberrant forms here described are not the result of irregularities in chromosome-distribution.

7. The aberrant forms are probably referable to *Sphaerocarpos Donnellii*.

The writer wishes to express his sincere appreciation and thanks to Professor Charles E. Allen, at whose suggestion and under whose direction this work was done.

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——. 1919. The basis of sex inheritance in *Sphaerocarpos*. Proc. Amer. Philos. Soc. 58: 289-316.  
Schacke, Martha A. 1919. A chromosome difference between the sexes of *Sphaerocarpos texanus*. Science, n. ser. 49: 218, 219.

#### DESCRIPTION OF FIGURES

##### PLATE XXXII

Portions of cultures of clones of various character. The plants are shown considerably enlarged, but not in exactly the same proportion in different photographs.

FIG. 1. Typical male clone (19.21).

FIG. 2. Polycladous female clone (20.267).

FIG. 3. Typical female clone (19.24).

FIG. 4. An entirely sterile portion of a semi-sterile male clone (R27B).

FIG. 5. Polycladous male clone (21.150). The spherical white bodies are antheridia.

##### PLATE XXXIII

The figures were drawn with a Zeiss compensating ocular no. 18 and a 2-mm. Zeiss apochromatic objective, N. A. 1.40, at table level with the aid of a camera lucida. With a tube length of 160 mm., a magnification of about 3,800 was obtained.

FIGS. 1-3. Chromosome groups in the apical meristem of the polycladous female clone (21.56) which was observed to bear occasional archegonia.

FIG. 4. Chromosome group in the apical meristem of a female polycladous clone on which archegonia were never observed.

FIG. 5. A metaphase stage in the apical meristem of a polycladous female.

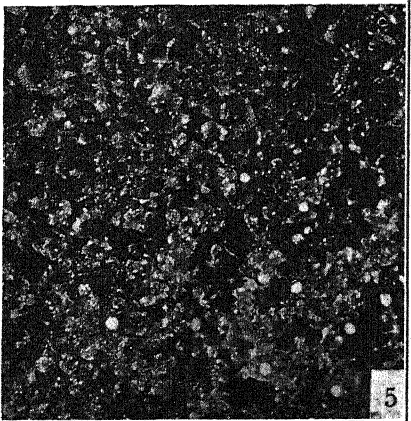
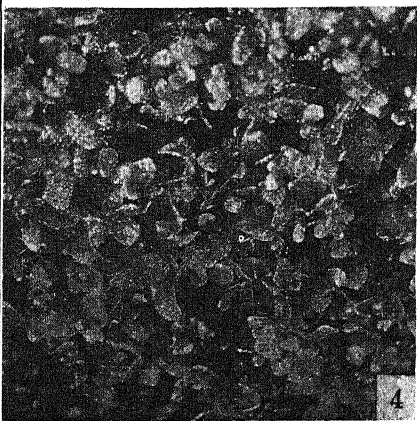
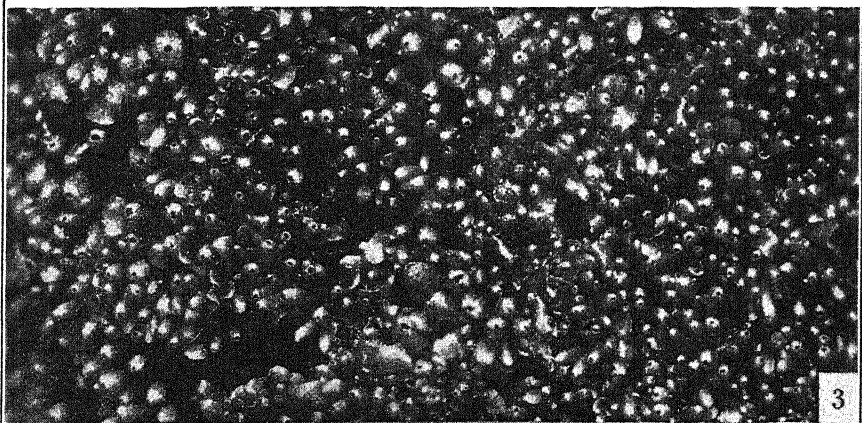
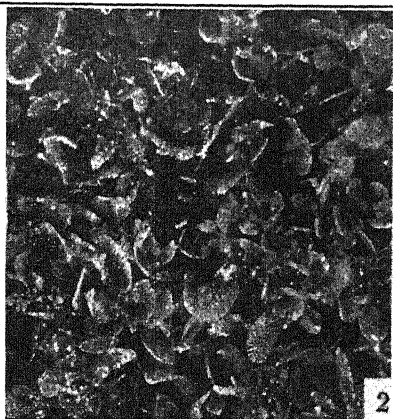
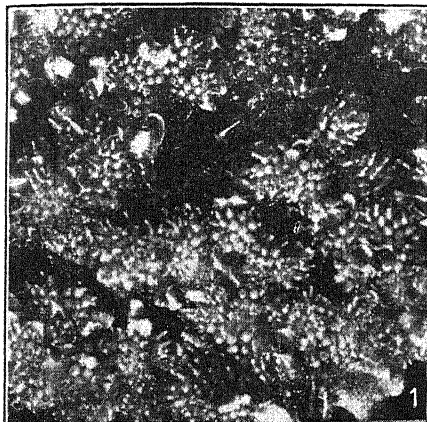
FIGS. 6, 7. Chromosome groups from developing antheridia of polycladous males.

FIG. 8. Chromosome group from the meristem of a polycladous male.

FIG. 9. Chromosome groups in spermatogenous cells of a polycladous male.

FIGS. 10, 11. Chromosome groups from a typical male.

FIGS. 12, 13. Anaphase groups in an antheridium of a semi-sterile male.



WOLFSON: ABERRANT FORMS OF SPHAEROCARPOS

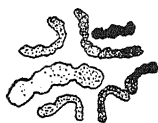




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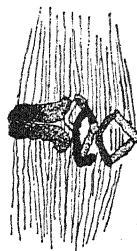
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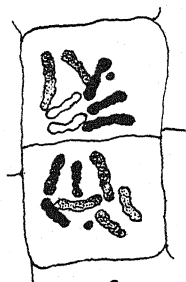
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9



10



11



12



13





## THE EMBRYOGENY OF *PASTINACA SATIVA*

FLOYD E. BEGHTEL

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During the last half century our knowledge of the embryogeny of the angiosperms has been extended to include a great many species well distributed among numerous families of both monocotyledons and dicotyledons. It is to be expected that our knowledge will advance most rapidly in those families in which the material proves to be most favorable. Numerous attempts have been made to trace the development of various species representative of the family Umbelliferae, but the small size of the ovule, together with certain mechanical difficulties incident upon imbedding and sectioning, have prevented any of these investigations reaching a stage which warrants publishing, with the exception of a recent work by Jurica (16).

The present investigation was begun in 1916 in the botanical laboratory at Indiana University under the direction of Dr. D. M. Mottier, and for the past six years has been continued in the botanical laboratory at the University of Cincinnati under the direction of Dr. H. M. Benedict. I wish to acknowledge my indebtedness to Dr. Mottier for suggesting the problem and for his generous aid in the pursuit of the study, and to Dr. Benedict for his invaluable aid during the latter years of the investigation.

*Pastinaca sativa* L., a species introduced from Europe but widely escaped from cultivation, was chosen for material, supplemented by other species available in southern Ohio. Flemming's solution was employed as a fixative, and a corrosive sublimate-acetic acid fixative gave good results in a few cases. Sections were cut six to ten microns, and stained on the slide with Flemming's triple stain.

### FLORAL DEVELOPMENT

The individual flowers arise as club-shaped masses of meristematic cells from the broad surface of the developing axis of the umbel. The rounded end of this structure soon broadens, and the primordia of petals, stamens, and carpels appear in the order named (Pl. XXXIV, fig. 1). (Sepals are obsolete but are occasionally seen in the material, as in figure 3. The order of their appearance could not be determined.) The two carpels begin their development as outgrowths from the meristematic surface. Each carpel is semilunar in shape, and as they unite along their inner margins they form an arch over a single ovarian cavity. Prior to the union

of these carpels their inner edges are sharply incurved, so that union is accomplished by the outer surface some distance from the edge. Thus the cavity of the ovary contains the edges of the carpels which by growth, chiefly from the base, finally divide this single cavity into two cells, each containing the two reflexed edges of a carpel (figs. 3, 4). From each of these edges an ovule begins to develop, a fact reported by Coulter and Rose (8) in 1888. All of these four ovules frequently develop to the condition in which the archesporium is distinguishable, but one in each cell degenerates before setting up that series of changes which leads to the production of the megaspore.

### ARCHESPORIUM

When the archesporium is first distinguishable, the ovule consists of a club-shaped organ attached by a rather slender funiculus to the inner wall of the ovarian cavity, in its apical portion, the ovule extending backward (fig. 8). The mature ovule is completely anatropous. It begins its development straight, and as growth continues it turns outward toward the exterior wall of the ovary and continues to a position of complete anatropy in which the integument on the side next the funiculus is scarcely recognizable.

The archesporium consists of a group of cells numbering from three to seven, which are easily distinguished by their staining reactions, their large nuclei, and especially by their form (figs. 7, Pl. XXXIV; 14, Pl. XXXV). They completely occupy the end of the nucellus, forming a mass almost spherical in shape, in which each cell borders upon the nucellar wall and also upon the adjacent cells of the nucellus which lie in the line of food-conduction from the chalaza. As a result of this arrangement, each cell is elongated in the direction of the long axis of the nucellus, those in the center being longer.

Sporogenesis in both the anthers and the ovules of angiosperms has now been investigated in a great many genera. While the archesporial tissue in the microsporangium is quite constantly a row, a plate, or a mass of cells, it was for a long time reported to consist of a single cell in the megasporangium. Strasburger (28) seems first to have reported a multicellular archesporium in the genus *Rosa* in 1879, although several other cases were reported the same year by Jönsson (15). Since this time a considerable number of genera have been investigated in which the archesporium sometimes consists of more than one cell.

Our present knowledge of this important matter is summarized in table 1.

TABLE I.

Family and Species	No. of Arch. Cells	Investigator
<b>Monocotyledoneae</b>		
<b>Butomaceae</b>		
<i>Butomus umbellatus</i> . . . . .	2-4	Holmgren (13)
<b>Araceae</b>		
<i>Aglaonema</i> . . . . .	2-3	Campbell (3)
<i>Arisaema triphyllum</i> . . . . .	2-4	Mottier (23)
<i>Nepthytis liberica</i> . . . . .	2-many	Campbell (3)
<b>Liliaceae</b>		
<i>Lilium philadelphicum</i> . . . . .	3-5	Coulter and Chamberlain (7)
<i>Ornithogalum pyrenaicum</i> . . . . .	2	Guignard (12)
<i>Smilacina racemosa</i> . . . . .	2	McAllister (21)
<b>Orchidaceae</b>		
<i>Calopogon pulchellus</i> . . . . .	2	Pace (27)
<i>Gastrodia</i> . . . . .	2	Kusano (18)
<b>Dicotyledoneae</b>		
<b>Casuarinaceae</b>		
<i>Casuarina suberosa</i> . . . . .	numerous	Treub (31)
<i>C. rumphiana</i> . . . . .	numerous	Treub (31)
<i>C. glauca</i> . . . . .	numerous	Treub (31)
<b>Piperaceae</b>		
<i>Chloranthus chinensis</i> . . . . .	numerous	Armour (1)
<i>C. officinalis</i> . . . . .	numerous	Armour (1)
<i>Peperomia hispidula</i> . . . . .	numerous	Johnson (14)
<b>Salicaceae</b>		
<i>Salix glaucophylla</i> . . . . .	2-3	Chamberlain (4)
<i>Populus tremuloides</i> . . . . .	2-3	Chamberlain (4)
<b>Juglandaceae</b>		
<i>Juglans cordiformis</i> . . . . .	numerous	Karsten (17)
<i>J. regia</i> . . . . .	several	Bensen (2)
<b>Betulaceae</b>		
<i>Betula alba</i> . . . . .	numerous	Bensen (2)
<i>Carpinus betulus</i> . . . . .	numerous	Bensen (2)
<i>Corylus avellana</i> . . . . .	several	Bensen (2)
<b>Fagaceae</b>		
<i>Castanea vulgaris</i> . . . . .	5-8	Bensen (2)
<i>Fagus sylvatica</i> . . . . .	numerous	Bensen (2)
<i>Quercus velutina</i> . . . . .	numerous	Conrad (5)
<b>Loranthaceae</b>		
<i>Loranthus sphaerocarpus</i> . . . . .	2	Treub (30)
<i>Viscum articulatum</i> . . . . .	4-many	Treub (30)
<b>Santalaceae</b>		
<i>Thesium</i> . . . . .	several	Coulter and Chamberlain (7)
<b>Phytolaccaceae</b>		
<i>Phytolacca decandra</i> . . . . .	2	Lewis (20)
<b>Ranunculaceae</b>		
<i>Caltha palustris</i> . . . . .	numerous	Mottier (24)
<i>Delphinium tricornis</i> . . . . .	2-many	Mottier (24)
<i>Helleborus cupreus</i> . . . . .	3	Coulter and Chamberlain (7)
<i>Ranunculus abortivus</i> . . . . .	2	Coulter (6)
<i>R. multifidus</i> . . . . .	2-3	Coulter (6)
<i>R. septentrionalis</i> . . . . .	2-13	Coulter (6)

TABLE I (continued)

Family and Species	No. of Arch. Cells	Investigator
Cruciferae		
<i>Capsella bursa-pastoris</i> . . . . .	2	Guignard (12)
Saxifragaceae		
<i>Astilbe japonica</i> . . . . .	several	Webb (35)
Rosaceae		
<i>Alchemilla acutangula</i> . . . . .	numerous	Murbeck (25)
<i>A. alpina</i> . . . . .	numerous	Murbeck (25)
<i>Agrimonia</i> . . . . .	numerous	Fischer (9) <sup>1</sup>
<i>Cydonia</i> . . . . .	numerous	Fischer (9)
<i>Eriobotrya japonica</i> . . . . .	3	Guignard (12)
<i>Geum</i> . . . . .	numerous	Fischer (9)
<i>Rosa livida</i> . . . . .	several	Strasburger (28)
<i>Rubus</i> . . . . .	numerous	Fischer (9)
<i>Sanguisorba</i> . . . . .	numerous	Fischer (9)
Linaceae		
<i>Linum usitatissimum</i> . . . . .	3	Jönsson (15)
Euphorbiaceae		
<i>Euphorbia procera</i> . . . . .	4-5	Modilewski (22)
Cistaceae		
<i>Helianthemum</i> . . . . .	several	Fischer (9)
Onagraceae		
<i>Lopezia coronata</i> . . . . .	4-5	Täckholm (29)
<i>Oenothera lamarckiana</i> . . . . .	2	Geerts (11)
Umbelliferae		
<i>Pastinaca sativa</i> . . . . .	3-8	Beghtel
Asclepiadaceae		
<i>Asclepias tuberosa</i> . . . . .	3-more	Frye (10)
Caprifoliaceae		
<i>Adoxa moschatellina</i> . . . . .	several	Lagerberg (19)
Campanulaceae		
<i>Lobelia syphilitica</i> . . . . .	2	Jönsson (15)
Compositae		
<i>Chrysanthemum leucanthemum</i> . . . . .	several	Jönsson (15)
<i>Pyrethrum balsaminatum</i> . . . . .	3	Ward (33) <sup>1</sup>

It should be stated that perhaps not all the papers referred to in connection with this list represent equal care in determining the exact limits of the archesporium. These limits are not always sharply marked out, and some of the works referred to date back to a time when the exact meaning of the term *archesporium* had not yet been carefully defined. However, only those species have been included in which the descriptions or drawings leave little doubt of the existence of more than one archesporial cell.

The occurrence of an archesporium of several or many cells in the ovule has come to be looked upon as a primitive character among the angiosperms, while the single hypodermal archesporial cell terminating an axial row in the nucellus is considered a derived character, reaching its greatest

<sup>1</sup> Original paper not available.

expression in the more highly specialized groups of both monocotyledons and dicotyledons. Thus, Coulter and Chamberlain (7) say that "The temptation is strong to consider the many-celled archesporium as a primitive feature of the dicotyledons." Campbell (3), in a paper dealing with the Araceae, says: "We may conclude, then, that the Araceae are really a primitive family of monocotyledons. . . . The not infrequent occurrence of a multicellular archesporium . . . points to such a conclusion." Also Pace (27), in discussing the multicellular archesporium, says: "It seems best to regard it as a primitive character that has been retained, or at least not entirely eliminated."

A study of the accompanying table indicates that the accumulated information on the question of a multicellular archesporium necessitates a careful reconsideration of the value of this character in determining the relative position of large groups in any phylogenetic scheme. It is still true that the majority of species reported belong to families recognized as among the more primitive, but the occurrence of an archesporium of more than one cell in such highly specialized families as the Compositae, Campanulaceae, Caprifoliaceae, and Umbelliferae can not be disregarded. As the number of genera reported from these families increases, the character in question certainly becomes less significant phylogenetically.

Another fact of considerable importance which has not been indicated in the table is the relative frequency of the occurrence of a multicellular archesporium in those genera where it has been observed. In *Calopogon* (Pace, 27) it was observed but once, in *Lilium philadelphicum* (Coulter and Chamberlain, 7) twice, in *Phytolacca* (Lewis, 20), *Gastrodia* (Kusano, 18), *Salix* (Chamberlain, 4), and *Asclepias* (Frye, 10) it is reported as occasionally met with, while in *Quercus* (Conrad, 5), *Pastinaca*, and many genera of the Rosaceae its presence seems to be the rule. In *Pastinaca* the author has examined a great number of ovules in the archesporial condition, and no instance of a single archesporial cell was found among this number. Any extensive comparisons of this kind are impossible, as there is great variation among the investigators as to the number of individuals examined and records are very incomplete.

#### PARIETAL TISSUE

Parietal tissue has been entirely suppressed in *Pastinaca*, and the cells of the archesporium are potentially spore mother cells. The failure of the archesporial cell or cells to divide in the production of a parietal layer has previously been pointed out in the family by Coulter and Chamberlain (7) in the genus *Sium*; and Jurica (16), in a recent publication, has figured *Sium cicutaefolium* and *Eryngium yuccifolium*. Although no statement is made in the discussion, the figures indicate clearly that no parietal tissue is developed in these species. It becomes increasingly evident, therefore, that the suppression of the parietal tissue, long known to be common

among certain of the less highly specialized groups, is also common in the Umbelliferae, which are representative of the higher Archichlamydeae.

The failure to produce parietal cells, which commonly contribute to the wall of the nucellus, accounts, at least in part, for the meager development of this nucellar wall. At the time that the archesporium is first distinguishable, it is surrounded by a single layer of cells constituting the nucellar wall (fig. 14, Pl. XXXV). These cells divide anticlinally only, and thus keep pace with the development of the sporogenous tissue without increasing the number of layers of the wall. This development, however, is not extensive, and the wall soon begins to break down. By the time the embryo sac is mature the wall has entirely disappeared, leaving the embryo sac surrounded only by the integument (fig. 20). The nucellar cap often seen in the micropylar end is quite wanting.

The layer of tapetum often found in the megasporangium is the product of the parietal tissue, and is consequently wanting in *Pastinaca*. Its nutritive function seems to be taken over by a mass of cells of the nucellus which remain back of the developing sporogenous tissue. This we may call the "nutritive tissue" (fig. 20). This nutritive tissue continues to degenerate as the development in the spore chamber proceeds, and no doubt contributes largely to the nourishment of the female gametophyte.

#### FATE OF THE MEGASPORE MOTHER CELL

The multicellular condition of the archesporium of the megasporangium is of peculiar interest because it represents a type of natural selection among cells each of which is potentially a megaspore mother cell, and one or more of which is destined to produce the megaspore and thus subsequently to play an important part in the ultimate development of the new sexually formed individual.

Unfortunately, few investigators have followed the development far enough to determine the fate of the megaspore mother cells in those cases in which more than one archesporial cell is produced. In those species in which this feature is but rarely met with such a study would be difficult; however, where it seems to be constant further study is very desirable.

In a number of species in which the multicellular archesporium has been found only a single embryo sac is formed, if we may judge from the information at hand: *Chloranthus* (Armour, 1), *Caltha* (Mottier, 24), *Lopezia* (Täckholm, 29), *Pastinaca*, and *Phytolacca* (Lewis, 20). In some other species the condition varies widely; thus, in *Smilacina* (McAllister, 21) two instances of two fully formed embryo sacs in an ovule were found; in *Casuarina* (Treub, 31) a number of tetrads are formed and several embryo sacs begin to develop but only one persists; in *Salix* (Chamberlain, 4) more than one embryo sac develops; in *Quercus* (Conrad, 5) one instance of two four-celled embryo sacs was found; in *Ranunculus* several may reach the four-celled stage; and in *Astilbe* (Webb, 35) several may begin

development. *Alchemilla* (Murbeck, 25) matures as many as four embryo sacs none of which is typical, while in *Astilbe* several mother cells may begin development. In *Euphorbia* (Modilewski, 22) four tetrads may be produced, while in *Nephthytis* (Campbell, 3) more than one embryo sac matures and in *Gastrodia* (Kusano, 18) each of two mother cells produces a normal embryo sac. These examples will serve to indicate the great irregularity in the time at which the selection among archesporial cells or their progeny is carried on as well as to show that in certain species, at least, the selection is never completed.

A number of genera have been reported in which the condition in the archesporium is unknown but in which two or more embryo sacs are known to develop in one ovule, or two or more embryos in one seed. It must be borne in mind that these facts do not necessarily indicate that the condition is the result of incomplete selection among previously existing archesporial cells, as the accessory embryo sac or embryo may have arisen in some other way. It is highly probable, however, that in these genera further study will reveal an archesporium of more than one cell. A more complete investigation of such genera is very important.

#### SELECTION IN PASTINACA

In *Pastinaca*, when the first changes leading to the heterotypic division in the mother cells are first noticeable, it is evident that processes of selection are already under way. Certain cells, usually the larger and more centrally located ones, first show mitotic activity, and the majority of the others may never institute such changes (fig. 11, Pl. XXXIV). There can be little doubt that the chief determining factor here is access to the available food supply. Those cells that are first able to reach a condition of food storage and organization which enables them to enter upon mitosis maintain a decided advantage over the others. In a large number of ovules examined, never more than three cells were found to have entered upon mitosis and in such cases one cell is always considerably in advance of the others. Figure 15, Plate XXXV, represents a cross section of a nucellus in which four of six archesporial cells have reached an advanced stage of deterioration, whereas, of two remaining, one is still in the resting condition while the other has entered upon nuclear division. A very large number of ovules containing the mature embryo sac have been examined and in no case has more than one embryo sac been found in an ovule. It is evident, therefore, that selection is speedily carried to the limit and that all but one of the elements are eliminated.

The first division of the megaspore mother cell is in every way typical of the heterotypic division. The synaptic knot is very compact and is followed by segmentation of the spireme thread. The chromosomes are short and thick, occurring in pairs. The spindle is quite complete (fig. 17). Subsequent steps follow the usual course of the reduction division. The

small size of the chromosomes and of the spindle, together with the difficulty of obtaining these stages which limits the number of good preparations, makes counting of the chromosomes very difficult and uncertain. The haploid number is probably eight.

The second division follows rapidly after the first, and subsequent development to the maturity of the embryo sac is accomplished in a remarkably short time. Great difficulty was experienced in obtaining preparations showing these steps, and, although a large amount of material was examined, it has been impossible to demonstrate beyond a doubt the production of a complete tetrad. It is known, however, that in both daughter cells, which are of approximately the same size, the second division is initiated at about the same time. It is possible that the division in the inner daughter cell is never completed, for very soon after the appearance of the spindles the entire sporogenous cavity of the ovule presents a condition very difficult to fix in such a way that clear preparations are possible. The tissue of the nucellus degenerates rapidly below the spore chamber, and several large vacuolate cells appear to occupy the cavity. Among the degenerating cells of the nucellar tissue at the chalazal end of the megaspore chamber are frequently to be seen three or more darkly staining, irregular masses believed to be the remains of the three inner megasporocytes together with those cells which have originated from other mother cells that did not degenerate before division. It should be borne in mind that at this time there is present in the spore cavity a great amount of degenerating tissue derived from numerous mother cells, and the whole nucellus is so much clouded that repeated efforts have failed to produce clear preparations.

It appears that the outer megaspore invariably functions, a condition that is an exception rather than the rule. Numerous cases have been reported in which the outer of three or four megasporocytes may function at times. Such a condition has been reported by Vesque (32), Treub (30), and Oliver (26). In *Pastinaca* no exception to the development of the outer megaspore has been noted among a great number of cases examined.

The developing megaspore penetrates rapidly and deeply into the tissue of the nucellus in the direction of the chalaza. The single layer of epidermal cells that composed the wall of the sporangium also breaks down, and the megaspore enlarges rapidly. When the eight-nucleate stage of the embryo sac is reached it occupies the entire space within the integument (fig. 20). There remains of the nucellar tissue only the small basal portion previously referred to as the nutritive tissue. Certain portions of this tissue still indicate by their staining reactions that degeneration is not yet complete.

#### THE OVULE

It has been stated by Coulter and Chamberlain (7) that the development of a single integument is characteristic of the Umbelliferae. This statement



holds for *Pastinaca*, as here only one integument is produced. The first indication of the integument appears at about the time that the sporogenous tissue is first distinguishable. It appears as a ridge-like swelling about the base of the nucellus (fig. 14). As is common in ovules in which a single integument is produced, that organ is thick and fleshy. The nucellus is completely enclosed by the developing integument at about the time that the tetrad of megaspores is produced.

#### THE EMBRYO SAC

The embryo sac is of the usual eight-nucleate type. The antipodals are small and lie within a depression of the tissue referred to as the nutritive tissue (fig. 20).

The flower of *Pastinaca* is completely epigynous, and the fruit is defined by Warming (34) as a schizocarp composed of two mericarps. The fruit is very much compressed laterally, bearing two free styles which unite at their base into a large nectary or stylar foot. This stylar foot develops early, and by the time the anthers begin to mature it secretes a nectariferous fluid. At this time the styles are very short and have not yet developed stigmatic surfaces. It is about five days after the first stamens of a flower begin to dehisce that the stigmas of the same flower are ready to receive pollen. The flowers of the outermost whorl in an umbel mature first. These are followed by those of the next whorl within, which shed their pollen at the time that the stigmas of the flowers of the outer whorl are receptive. In this manner cross-pollination in the umbel is accomplished. This type of cross-pollination seems to be the rule, while that between umbels is not common. This condition is shown by the failure of the last whorl of flowers at the center of the umbel to develop because of failure to be pollinated.

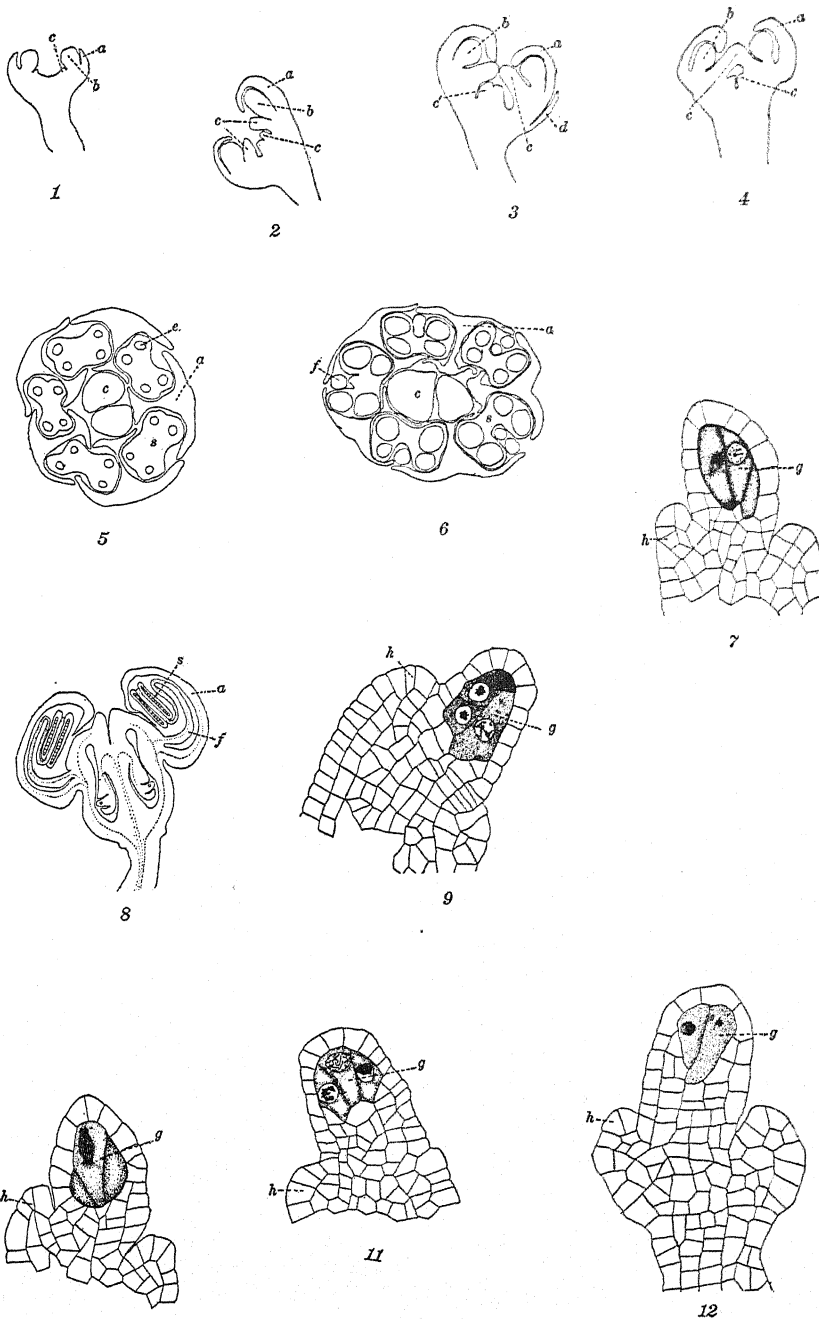
Preparations showing fertilization of the egg were not secured, although the pollen tube was traced through the tissues of the style and into the tissues beneath the stylar foot. There is no indication that the history of fertilization and the development of the embryo and of the endosperm vary in any way from the usual form described in other dicotyledonous families.

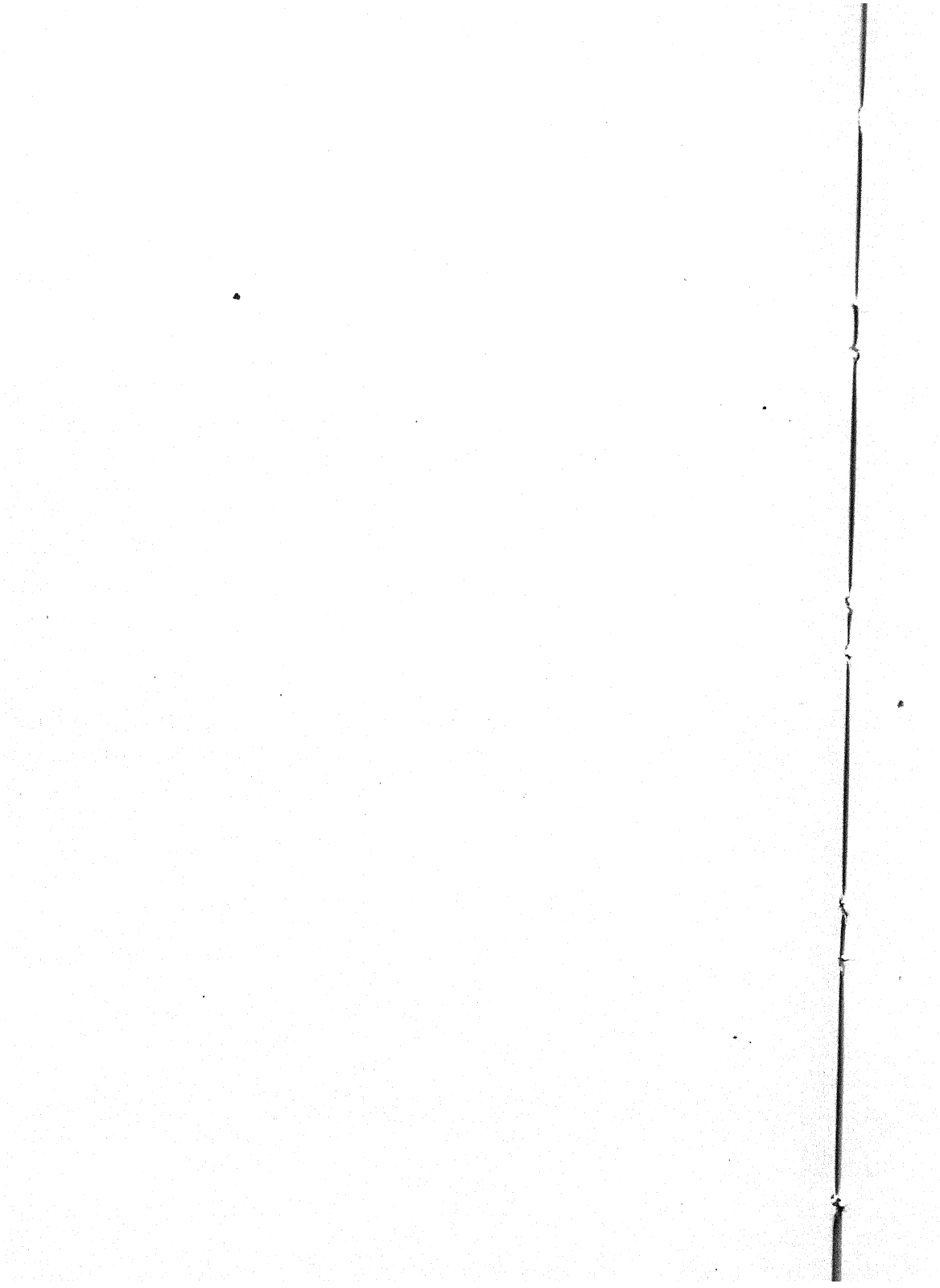
#### SUMMARY

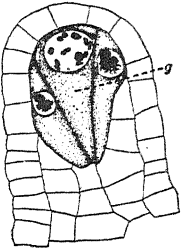
1. Four ovules begin development in the ovary of *Pastinaca*, two of which early degenerate, leaving one in each cell.
2. The archesporium consists of a mass of three to seven cells.
3. A review of our present knowledge of the multicellular archesporium in the megasporangium of angiosperms indicates that this character is of little value in determining phylogenetic relationships.
4. No parietal tissue is produced.
5. The outer of three or four megaspores functions.
6. Only one embryo sac matures, and it is typically eight-nucleate.
7. A single thick, fleshy integument is produced.

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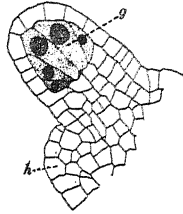
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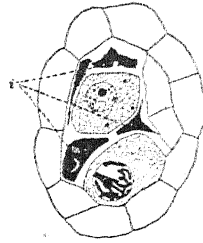




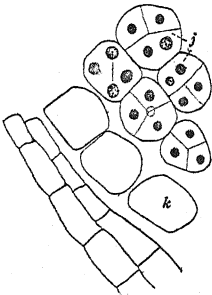
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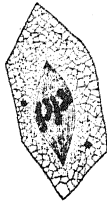
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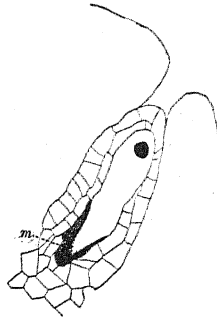
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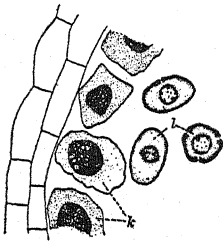
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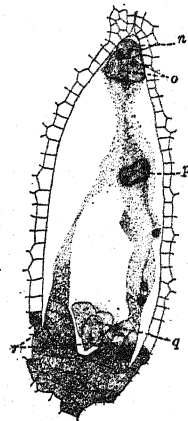
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#### EXPLANATION OF PLATES

*a*, petals; *b*, stamens; *c*, carpels; *c'*, carpels united along margin and developing from base of cavity; *d*, rudimentary sepal; *e*, archesporial tissue in anther; *g*, archesporial tissue in ovule; *h*, integument; *i*, degenerating mother cells; *j*, microspore tetrads; *k*, tapetum; *l*, young pollen grains; *m*, degenerating tissue; *n*, egg; *o*, synergids; *p*, polar nuclei; *q*, antipodals; *r*, nutritive tissue.

#### PLATE XXXIV

- FIG. 1. Longitudinal section of a young flower.  
FIG. 2. Same as figure 1, but older.  
FIG. 3. Same as figure 2, showing rudimentary sepal.  
FIG. 4. Longitudinal section of a flower of the same age as in figures 2 and 3, but cut with the plane of sectioning rotated ninety degrees.  
FIG. 5. Cross section of a flower, showing the arrangement of parts at the time of the appearance of the archesporium in the anther.  
FIG. 6. Same as figure 5, but nearer to the base of the flower.  
FIG. 7. Longitudinal section of the nucellus, showing the archesporium and nucellus.  
FIG. 8. Longitudinal section of an entire flower of the same age as that from which figure 7 was drawn. Dotted lines represent vascular strands and indicate lines of development of the flower.  $\times 100$ .

FIGS. 9-12. Examples showing multiple archesporium, varying slightly in stage of development as indicated by the condition of the nuclei.  $\times 950$ .

#### PLATE XXXV

- FIGS. 13, 14. Further examples of multiple archesporium. Figure 13,  $\times 1200$ . Figure 14,  $\times 950$ .  
FIG. 15. Cross section of nucellus showing degenerating mother cells.  
FIGS. 16, 19. Stages in the development of the microspores.  
FIG. 17. Spindle of the heterotypic division in the megaspore mother cell.  $\times 1750$ .  
FIG. 18. Longitudinal section of nucellus, showing germinating megaspore.  
FIG. 20. The mature embryo sac.  $\times 500$ .

# THE GEOGRAPHICAL DISTRIBUTION IN NORTH AMERICA OF POISON IVY (*RHUS TOXICODENDRON*) AND ALLIES

JAMES B. McNAIR

(Received for publication September 1, 1924)

The following table compiled from lists of herbarium specimens shows that poison ivy (*Rhus Toxicodendron* L.) and its allies occur in every province of Canada bordering on the United States, in every state in the United States, in most of the states of Mexico, and in the Bermuda Islands.<sup>1</sup> They are found between 15° and 50° north latitude and from 60° to 125° longitude. The area occupied includes altitudes from sea level to approximately 6,000 feet above sea level. The desert and high mountain regions are free from the plants.

Through the courtesy of W. C. Henderson, the author is enabled to add a list of places where birds have been found with poison-ivy seeds in their stomachs. These locations correspond with the places where the plants grow, as shown by the herbarium lists.

No attempt has been made to revise the lists of specimens as to various species or homologous species or sub-species beyond changing, for the sake of uniformity, the genus name *Toxicodendron* to *Rhus*. With this exception the lists have been copied as sent. In future publications the author expects to undertake a review of the taxonomy of *Rhus Toxicodendron* L. and its allies.<sup>2</sup>

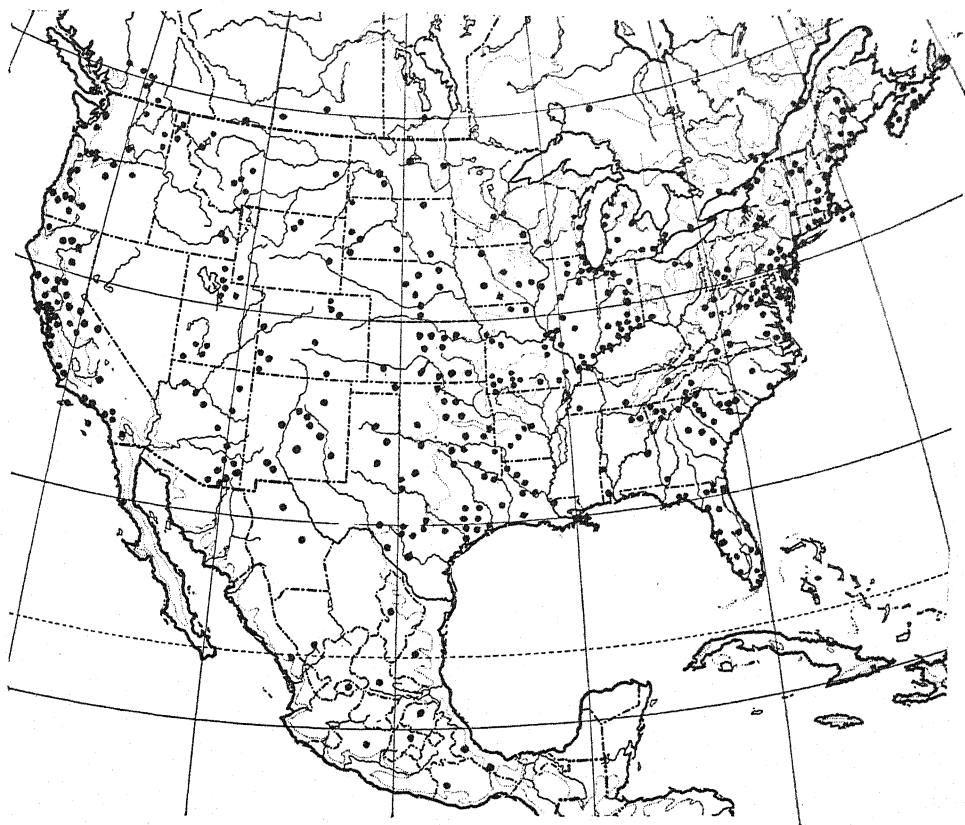
The author desires to express his grateful appreciation for the coöperation of Marcus E. Jones, Pomona College, Claremont, Cal.; M. O. Malte, Department of Mines, Victoria Memorial Museum, Ottawa; John Davidson, University of British Columbia, Vancouver; W. C. Henderson, Bureau of Biological Survey, U. S. Department of Agriculture, Washington; S. F. Blake, Bureau of Plant Industry, U. S. Department of Agriculture, Washington; W. R. Maxon, U. S. National Herbarium, Washington; D. C. Davies, B. E. Dahlgren, Francis Macbride, and Miss Edith M. Vincent, Field Museum of Natural History, Chicago; B. L. Robinson, Gray Herbarium, Cambridge, Mass.; Alfred Rehder, Arnold Arboretum, Jamaica Plain, Mass.; J. M. Greenman and Carl Epling, Missouri Botanical Garden, St. Louis; P. A. Rydberg and Mrs. Palmyra de C. Mitchell, New York Botanical Garden, New York; Francis W. Pennell, Academy of Natural Sciences, Philadelphia.

<sup>1</sup> Additional locations in California, Oregon, Washington, and British Columbia may be found in the American Journal of Botany, 8: 127-137. 1921; and in McNair, J. B., *Rhus dermatitis*, its pathology and chemotherapy. Chicago, 1923.

<sup>2</sup> The taxonomy of poison ivy with a note on the origin of the generic name. Field Museum of Natural History. Botanical Series. 4: 55-76. 1925.



The letters following the names of locations indicate the herbaria in which the specimens are to be found. These letters and their meanings are: A, Arnold Arboretum; B, University of British Columbia; C, Canadian



TEXT FIG. 1. Map showing sources of herbarium specimens of *Rhus Toxicodendron* L.

National Herbarium; F, Field Museum of Natural History; G, Gray Herbarium; M, Missouri Botanical Garden; N, New York Botanical Garden; P, Pomona College; S, Philadelphia Academy of Natural Sciences; U, U. S. National Herbarium.

#### CANADA

ALBERTA. *R. Rydbergii* Small: Rosedale, M. E. Moodie, N; Rosedale, U. *R. Toxicodendron* L.: Castellated Rocks, Macoun, C; Milk River; Rosedale, 2200-2500 ft., F.

MANITOBA. *R. Toxicodendron*: Ameme, N; Criddle, A; Brandon, Macoun, C; Dufferin, G. M. Dawson, C; Delta, J. B. Hales, A.

BRITISH COLUMBIA. *R. Rydbergii*: Armstrong, J. Davidson, B; Bos-

well, J. Davidson, B; Spence's Bridge, J. Davidson, B; Spence's Bridge, J. Macoun, N. *R. Toxicodendron*: Deer Park, Macoun, C; Lower Arrow Lake, Hot Springs, Dawson, C; Arrow Lake, Lake Osoyoos, J. M. Macoun, C; Spence's Bridge, Macoun, C; Spence's Bridge, C. F. Neucombe, F.

NEW BRUNSWICK. *Toxicodendron vulgare* Mill.: Jemseg, G. U. Hay, C.

NOVA SCOTIA. *R. radicans* L.: Ferrona Junction, C. B. Robinson, N. *R. Toxicodendron*: Annapolis, Macoun, C; Argyle Head, Long and Linder, G; Bellevue Cove, J. Macoun, C; Big Internal, Cape Breton Island, Macoun, C; Bridgewater, Lunenburg Co., M. L. Fernald and B. Long, A, G, S; East Bridgewater, J. Macoun, C, L; East Jordan, Shelburne Co., Fernald and Long, G, S; Five Mile River, Hants Co., A. S. Peese and B. Long, G, S; Five River (Morris) Lake, Fernald and Long, A, G; Godfrey Lake, Fernald and Long, G; Harper Lake, Fernald and Long, G; Mahone Bay, L. Kady, C; New Germany, C. A. Hamilton, C; Port Bevis, Victoria Co., Fernald and Long, G, S; Shubenacadie Grand Lake, Halifax Co., Fernald and Bissell, G; Shubenacadie Grand Lake, Halifax Co., Fernald and Long, S; Springfield Junction, J. Macoun, C; Tusket, Long and Linder, G; Tusket (Vaughan) Lake, Fernald *et al.*, G; Yarmouth, Yarmouth Co., Pease and Long, G, S; Welshtown (Birchtown) Lake, Fernald and Long, G.

ONTARIO. *R. Toxicodendron*: Amherstburg, Macoun, C; Britannia, W. H. Harrington, C; Edmonton, U; Georgian Bay, A. G. Huntsman, C; Governor's Bay, Ottawa, Macoun, C; London, Burgess, C; Port Colborne, Macoun, C; Rockcliffe, Ottawa, J. Macoun, C; St. Thomas, J. Macoun, C; Williams, A. H. Richardson, A. *T. vulgare*: Niagara Falls, W. Scott, C; Pine Lake, Algonquin Park, Macoun, C; Queenston, Macoun, C; Wooler, Macoun, C.

QUEBEC. *R. Toxicodendron*: Hull, Fletcher, C; Hull, W. H. Harrington, C; Hull, J. Macoun, G; Isle of Orleans, J. G. Jack, A. *T. vulgare*: Montmorency Falls, Macoun, C, G.

SASKATCHEWAN. *R. Toxicodendron*: Bredenburgh, J. Macoun, C. W. Herriott; Cypress Hills, Macoun, C; Moose Jaw, Macoun, C.

## MEXICO

BAJA CALIFORNIA. *R. diversiloba* T. & G.: El Rosario, T. S. Brandegee, A.

CHIHUAHUA. *R. Toxicodendron*: Cajon Creek, U; San Diego, U; San Diego, 6000 ft., C. V. Hartman, A, F; San Luis Potosi, U; San Luis Potosi, 6000-8000 ft., C. C. Parry and Ed. Palmer, S. *R. phaseoloides* Greene: San Diego, C. V. Hartman, N.

CUICATLAN. *R. Toxicodendron*: Cuyameras, 2000 meters, Conzatti, F.

DURANGO. *R. eximium* Greene: Durango City, Palmer, N.

GUANAJUATO. *R. radicans*: Guanajuato, U.

GUERRERO. *R. radicans*: Omilteme, U.

HIDALGO. *R. radicans*: Ixmiquilpan, U.

- MEXICO. *R. radicans*: Mt. Ixtaccihuatl, U.  
MICHUACAN. *R. radicans*: Morelia, U; *R. Toxicodendron*: Morelia, Aresene, F.  
NUEVO LEON. *R. eximium*: Monterey, U. *R. quercifolia* (Michx.) Steud.: Monterey, Bro. Abbon, A.  
OAXACA. *R. radicans*: Reyes, U; San Miguel, Albarrados, U.  
SAN JUAN. *R. Toxicodendron*: Loma, Bro. G. Aresene, A.  
SINALOA. *R. radicans*: San Ignacio, U.  
SONORA. *R. phaseoloides*: Guadalupe, R. E. K. Smith, N; Sierra del Pajarito, Schott, N; Turucachi, C. V. H. Hartman, N.  
TAMAULIPAS. *R. eximium*: Victoria, U. *R. phaseoloides*: Victoria, 320 meters, Palmer, N.  
VERA CRUZ. *R. radicans*: Orizaba, U.

## UNITED STATES

- ALABAMA. *R. quercifolia*: Auburn, F. S. Earle, C. F. Baker, M; Blount Co., H. Eggert, M; Etowah Co., H. Eggert, M. *R. radicans*: Auburn, Lee Co., Earle and Baker, N. *R. Toxicodendron*: S. B. Buckley, M; Auburn, Earle and Baker, F, M, N; Bladon Springs, U; Blount, Eggert, N; Blount Co., H. Eggert, M; Blount Spring, Eggert, N; Etowah Co., H. Eggert, M; Mobile, U.  
ARIZONA. *R. diversiloba*: Bisbee, J. I. Carson, A; Flagstaff, 7000 ft., D. T. MacDougal, F, S; Fort Apache, P. S. Mayerhoff, F; Grand Canyon, C. F. Millsbaugh, F. *R. laetevirens* Greene: Santa Catalina Mts., Toumey, N; Tanner's canyon, R. C. Ebert, N. *R. phaseoloides*: Nogales, U; Santa Catalina Mts., U. *R. Rydbergii*: Bright Angel Canyon, U; Chiricahua Mts., U; Flagstaff, MacDougal, N; Ft. Huachuca, U; Navajo Reservation, Univ. of Arizona, A; Navajo Reserv., Univ. of Ariz., U; Paradise, U; Sabino Canyon, Santa Catalina Mts., A. Rehder, A; Santa Rita Mts., U; Santa Catalina Mts., J. W. Toumey, A. *R. Toxicodendron*: Catalinas, Jones, P; Chaperon Canyon, Chiricahua Mts., 7500 ft., J. C. Blumer, F; Chiricahua Mts., Blumer, M; Dos Cabezas Mts., Lemmon, G; Flagstaff, A. Rehder, A; Flagstaff, MacDougal, S; Fort Huachuca, Jones, P (observed); Fort Huachuca, E. Palmer, G; Huachuca Mts., D. Griffith, M; Lowell Mts., Parish, G; Mule Mts., Jones, P (observed); Santa Rita Mts., Jones, P; Rio Verde, E. Cones and E. Palmer, M; Rocky Mts., E. Hall and J. P. Harbour, F; Willow Spring, J. T. Rothrock, L.  
ARKANSAS. *R. quercifolia*: Fulton, B. F. Bush, A; Little Rock, E. J. Palmer, A; Pinnacle, Palmer, A. *R. Toxicodendron*: Fulton, U; Fulton, Bush, A, M; Hot Springs, Palmer, A; Pulaski Co., Hasse, N.  
CALIFORNIA. *R. diversiloba*: Alpine, U; Amador Co., U; Berkeley, W. L. Jepson, N; Big Chico Creek, A. A. Heller, A, N; Big Chico Creek, 250 ft., Butte Co., Heller, F; Black Mt., Santa Clara Co., A. D. E. Elmer, N; Black Mt., U; Blockman's Ranch, Mariposa Co., A. Eastwood, A;

Butte Co., Heller, N; Calaveras River, Gibbes, N; Chico, Heller, A; Chico, Palmer, P; Chico, U; Colusa, Eastwood, A; Contra Costa Co. near Clayton, U; Fort Tejon, Xantus de Vessly, N; Fort Tejon, U; Geysers, Torrey, N; Havilah, U; Ione, Eastwood, A; Little Chico, Butte Co., Mrs. C. Bruce, N; Little Chico Creek, U; Los Angeles, S. F. Blake, A; Los Gatos, Heller, A, F, N, S; Los Gatos, B. F. Leeds, F; Los Gatos, U; Marin Co., U; Mariposa Co., Hollick, N; Martinez, Bigelow, N; Mendocino, Mendocino Co., H. E. Brown, L, N; Mendocino, U; Monterey, Bigelow, N; Monterey, Hartweg, H; Monterey, U; Monterey Co., T. S. Brandegee, A; Mt. Tamalpais, O. Kuntze, N; Mt. Lowe, Los Angeles Co., C. S. Williamson, S; Murphy, Calaveras Co., Ben H. Smith, S; Napa Valley, Torrey, N; New Almaden, Torrey, N; Oroville, U; Oroville, 8 miles east, Heller, A; Oroville, 8 miles north, Heller, N; Oroville, Table Mt., 600 ft., Heller, L, S; Pasadena, M. E. Jones, A, M, P; Pasadena, U; Petrified Forest, Eastwood, A; Ross Valley, Eastwood, A; Valley of Sacramento River, Stillman, N; San Clemente Island, U; San Diego Co., E. Palmer, F; San Gabriel, Bigelow, N; Santa Barbara, Elmer, F, N; Santa Barbara, U; Santa Catalina Island, E. C. Knopf, L; Santa Clara Co., J. J. McMurphy, A; Santa Cruz Island, U; Santa Cruz Island, Brandegee, A; Santa Cruz Mts., U; Saratoga Springs, B. F. Leeds, F; Shasta Springs, Eastwood, A; Stanford University, C. F. Baker, N; Stanford University, McMurphy, N; Stanford University, U; Sulphur Mts., U; Sulphur Mt. Springs, L. Abrams, E. A. McGregor, N, A; Tres Piños, U; Ukiah, Eastwood, A; Ventura, Eastwood, A.

COLORADO. *R. Rydbergii*: Boulder Co., Tweedy, N; Ft. Collins, U; Ft. Collins, 5000 ft., Crandall, N; Grand Junction, A. Eastwood, A; Navajo Canyon, U; Norwood Hill, San Miguel Co., U; Pikes Peak, U; San Miguel Co., E. P. Walker, N; Trinidad, Dixon Canyon, N. *R. Toxicodendron*: Denver, Eastwood, F; Ft. Collins, U.

CONNECTICUT. *R. radicans*: New Haven, D. C. Eaton, N; *R. Toxicodendron*: Canaan, Fernald, G; Greenville, G. R. Lumsden, C; Middletown, S. B. Buckley, M; Oxford, E. B. Harger, G.

DELAWARE. *R. quercifolia*: Laurel, U. *R. Toxicodendron*: B. F. Leeds, L; Laurel, Commons, N, G; Laurel, C. S. Williamson, S; Ruthby, Albert Commons, S.

DISTRICT OF COLUMBIA. *R. Toxicodendron*: Brookland, Holm, G; Chesapeake Beach Junction, U; Washington, F. L. J. Boettcher, A, G; Washington, J. H. Painter, M; Washington, E. S. Steele, M.

FLORIDA. *R. Blodgettii* Kearney: Alva, A. S. Hitchcock, M; Jacksonville, A. H. Curtiss, M; Pine Key, Blodgett, N; Tampa Bay, P. H. Rolfs, M; *R. floridana* Mearns; Alva, Lee Co., Hitchcock, N; Camp Longuien, Small and Wilson, N; between Homestead and Camp Jackson, Small and Wilson, N; Miami, Small and Nash, N; Silver Palm School, Dade Co., Small, N. *R. quercifolia*: Suwanee Co., Hitchcock, M. *R. radicans*:

Everglade Keys, Dade Co., Small and Mosier, N; Hammock, Dade Co., Small and Mosier, N; Lake Okeechobee, Small, N; Tallahassee, N. K. Berg, N; *R. Toxicodendron*: Alva, Hitchcock, G; Columbia Co., Hitchcock, M; Brevard Co., Fredholm, G; Dade Co., U; Duval Co., U; Ft. Myer, T. G. Harbison, A; Gainesville, Joseph Crawford, S; Indian River, E. Palmer, M; Jefferson Co., Hitchcock, L; Lake City, U; Lake City, Hitchcock, F; Lee Co., Hitchcock, M; Levy Co., Hitchcock, F; Manatee River mouth, Rugel, G; Marco, Hitchcock, F; Myers, Hitchcock, F; Palm Beach, Hitchcock, F; Suwanee Co., Hitchcock, F, M; Tampa Bay, P. H. Rolf, F, M.

GEORGIA. *R. quercifolia*: Augusta, C. S. Sargent, A; Battle, Sargent, A; Bullock Co., R. M. Harper, M; Bullock Co., U; Lookout Mt., A. Ruth, M; Swinnett Co., U. *R. radicans*: Bainbridge, Decatur Co., Small, N; Jefferson Co., M. H. Hopkins, N. *R. Toxicodendron*: Augusta, Richmond Co., Small, N; Bainbridge, J. K. Small, A; Bullock Co., Harper, M, N; Habersham Co., Small, N. *R. Toxicodendron*: Jefferson Co., Hopkins, N; Lookout Mt., Ruth, M; Lookout Mt., Ruth, N; Milledgeville, Samuel Boykin, S; Whitfield Co., P. Wilson, N.

IDAHO. *R. Rydbergii*: Coeur d'Aléne, U; Hope, Kootenai Co., Sanberg, MacDougal, and Heller, N; Inkom, J. G. Jack, A. *R. Toxicodendron*: Pocatello, Jones, P (observed).

ILLINOIS. *R. microcarpa* (Michx.) Steud.: Pittsburgh, E. Douglass, M; *R. negundo* Greene: Starved Rock, LaSalle Co., L. Greenman and R. A. Dixon, N; *R. radicans*: Chicago, F; Edgebrook, Cook Co., H. F. Gates, F; Leyden, Cook Co., Gates, F; Romeo, L. M. Umbach, F. *R. Toxicodendron*: Balto Co., K. A. Taylor, F; Cohokia, H. Eggert, M; E. St. Louis, G. Engelmann, M; Edgebrook, Cook Co., Gates, F; Glencoe, M. Bross, F; Golconda, E. J. Palmer, A; Golconda, Pulaski Co., U; Joliet, H. C. Skeels, F; Lake Co., F. C. Gates, F; Liana, Hancock Tp., Hancock Co., F. C. Gates, F; Mounds, Palmer, A; Ottawa, Huett, G; Peoria Co., J. T. Stewart, F; Pittsburgh, Douglass, M; Springfield, Bobb, F; Starved Rock, LaSalle Co., J. M. Greenman, F, G; Stony Island, Cook Co., H. H. Smith, F, G; Tunnel Hill, Palmer, A; Waukegan, Lake Co., F. C. Gates, F; Winnebago Co., M. S. Bebb, F.

INDIANA. *R. radicans*: Wells Co., Deam, N. *R. Toxicodendron*: Aurora, C. C. Deam, A; Baden, Deam, A; Bloomington, Deam, A; Cannelton, Deam, A; Chesterton, Deam, A; Clarke, U; Cross Plains, Deam, A; Dayville, Deam, A; Edinburg, Deam, A; Elizabeth, Deam, A; Evansville, Deam, A; Gordon, Deam, A; Greencastle, U; Hanover, J. M. Coulter, F; Harrison, Wells Co., Deam, G; Ingalls, Madison Co., H. H. Smith, G; Lafayette, Deam, A; Lake Maxinkuckee, U; Lexington, Deam, A; Madison, Deam, A; Marion, Deam, A; Millers, U; Milltown, Deam, A; Riverdale, Deam, A; Solon, Deam, A; Spencer, Deam, A; Tremont Beach, Lake Michigan, Deam, A; Versailles, Deam, A; Wells Co., U; Wells Co., Deam, M; Whiting, O. E. Lansing, F; Yankeetown, Deam, A.

IOWA. *R. Toxicodendron*: Ames, A. S. Hitchcock, M; Ames, J. R. Campbell, M; Decatur Co., J. P. Anderson, M; Grinnell, Jones, P; Johnson Co., T. J. Fitzpatrick, F; Spirit Lake, B. W. Evermann, F; Shelby Co., Fitzpatrick, F.

KANSAS. *R. microcarpa*: Riley Co., J. B. Norton, M. *R. negundo*: Cowley Co., M. White, N; Riley Co., Norton, N. *R. radicans*: Manhattan, N. *R. Rydbergii*: Osborne City, U; Syracuse, Hamilton Co., C. H. Thompson, N. *R. Toxicodendron*: Arkansas City, E. J. Palmer, A; Downs, Palmer, A; Ellsworth, Palmer, A; Gypsum City, Palmer, A; Manhattan, M; Neodesha, Palmer, A; Osborne City, Shear, G; Riley Co., Norton, M; Rockport, E. Bartholomew, M; St. George, W. A. Kellerman, M; Syracuse, Thompson, G, M.

KENTUCKY. *R. Toxicodendron*: . . . C. W. Short, M.

LOUISIANA. *R. microcarpa*: Catalpa, West Feliciana Parish, Pennell, N. *R. quercifolia*: Chestnut, E. J. Palmer, A; Chopin, Palmer, A. *R. radicans*: Alexandria, C. R. Ball, N. *R. Toxicodendron*: . . . Hale, G; Alexandria, U; Alexandria, Ball, G, M; Alexandria, Josiah Hale, S; Lake Charles, U; Natchitoches, Palmer, A; New Orleans, R. S. Cocks, A; New Orleans, A. Fendler, M; Shreveport, C. S. Sargent, A.

MAINE. *R. Toxicodendron*: Fairfield, Somerset Co., Fernald and Long, G, S; Fort Fairfield, Fernald, G; Monticello, Aroostook Co., Fernald and Long, S; Mt. Desert Island, J. H. Redfield, M, S; Orono, U; Ox Cove, Washington Co., Fernald, G; Rockland, Knox Co., Fernald, G; Wells, York Co., Fernald and Long, S; Winn, Penobscot Co., Fernald and Long, S; Winthrop, E. L. Sturdevant, M.

MARYLAND. *R. quercifolia*: Salisbury, U. *R. Toxicodendron*: Annapolis, U; Annapolis, I. Tidstrom, A; Claiborne, U; Prince George Co., U; Savage Station, C. S. Williamson, S; Snow Hill, J. B. S. Norton, M.

MASSACHUSETTS. *R. radicans*: Ipswich, Morong, N; Oak Bluffs, F. C. Seymour, N. *R. Toxicodendron*: Bolton, C. H. Knowlton, S; Brookline, C. E. Faxon, G; Brookline, J. Robinson, A; Centerville, E. L. Williams, G; Falmouth, Barnstable Co., F. W. Pennell, S; Hyannis, Barnstable Co., Fernald *et al.*, G; Hyannisport, J. R. Churchill, G; Lexington, E. F. Williams, G; Martha's Vineyard, U; Martha's Vineyard, Oak Bluffs, F. C. Seymour, G; Middleboro, J. Murdock, F; Middlesex Fells, E. F. Williams, G; Nantucket, A. Rehder, A; New Salem, U; Nonquit, E. L. Sturdevant, M; South Hadley, U; West Roxbury, E. F. Williams, G; Weston, Fernald, G; Winchester, E. F. Williams, G; Woods Hole, A. A. Moore, F.

MICHIGAN. *R. microcarpa*: Belle Isle, Detroit, Farwell, N; Ludington, A. I. McClatchie, N. *R. Rydbergii*: Douglas Lake, Sheboygan Co., R. M. Harper, N. *R. Toxicodendron*: Agricultural College, U; Agricultural College, Skeels, G; Ann Arbor, J. Q. A. Fritchey, M; Cheboygan Co., U; Hamlin Lake, Ludington, Mason Co., R. W. Chaney, F; Ross, G. D. Jones, M; Saginaw Bay, C. K. Dodge, G; South Grand Rapids, C.

MINNESOTA. *R. Toxicodendron*: Anthony Park, J. H. Schuette, F; Ft. Snelling, U; Houston, Butters and Rosendahl, G; Minneapolis, A. P. Anderson, C; Piedmont, A. D. Pratt, F; St. Paul, St. Anthony Park, Schuette, G; Spring Grove, Rosendahl, G.

MISSISSIPPI. *R. Toxicodendron*: . . . E. Hilgard, M.

MISSOURI. *R. microcarpa*: Allenton, Letterman, M; Courtney, B. F. Bush, M; St. Charles Co., E. Douglass, M; St. Louis, G. Engelmann, M. *R. negundo*: Waco, Jasper Co., Palmer, N. *R. pubescens* Miller: Independence, Bush, F. *R. radicans*: Allenton, Letterman, N; St. Louis, Eggert, N. *R. Toxicodendron*: Allenton, U; Allenton, Letterman, M; Branson, E. J. Palmer, A; Carsonville, J. Q. A. Fritchey, M; Carthage, Palmer, A; Courtney, Bush, M; Dumas, Bush, A; Independence, Bush, A; Jasper, Bush, A; Joplin, Palmer, A; Kimmswick, F. Wislizenus, M; Larussell, Palmer, A; Montier, Bush, A; Neck City, Palmer, A; Noel, U; Noel, Bush, A; Old Orchard, L. H. Pammel, A; Prosperity, Palmer, A; St. Charles Co., Douglass, M; St. Louis, M. Craig, A; Sibley, Bush, A; Springfield, U; Swan, Bush, A; Swope Park, Kansas City, Bush, A; Valley Park, . . . , M; Van Buren, U; Van Buren, Palmer, A; Vulcan, Iron Co., H. H. Smith, F; Waco, Palmer, A; Watson, Palmer, A; Webb City, Palmer, A; Windsor Spring, M.

MONTANA. *R. Rydbergii*: Big Fork, B. T. Butler, N; Bozeman, J. W. Blankinship, M; Bozeman, 5000 ft., Blankinship, F; Cascade Co., U; Flathead Co., J. W. Jack, A; Flathead Lake, Mrs. J. Clemens, F; Glendive, U; Great Falls, U; Helena, F. W. Anderson, N; Helena, F. D. Kelsey, N; Rocky Canyon, 5000 ft., Blankinship, C; Rocky Canon, U. *R. Toxicodendron*: Bozeman (Rocky Canyon, 5000 ft.), Blankinship, S; Flathead Lake, Jones, P (observed).

NEBRASKA. *R. Rydbergii*: Kearney Co., Rydberg, N; Long Pine, U; Newcastle, Clements, N; Thomas Co., U. *R. Toxicodendron*: Franklin, W. A. Laybourne, M; Kearney Co., P. A. Rydberg, A; Newcastle, U; Ravenna, J. G. Jack, A. Rehder, A; Stanton, U.

NEVADA. *R. Rydbergii*: Wasatch, U.

NEW HAMPSHIRE. *R. Toxicodendron*: Hanover, U; Hanover, C. H. Hitchcock, C; Manchester, D. Smith, A; Warren, E. F. Williams, G.

NEW JERSEY. *R. littorales* Mearns: Barnegat Pier, Ocean Co., Mackenzie, N. *R. radicans*: New Brunswick, F. H. Blodgett, N; New Brunswick, F. L. Stevens, L; Spotswood, Middlesex Co., N. Taylor, N. *R. Toxicodendron*: Bennett, Cape May Co., Bayard Long, S; Bridgeton, S. S. Van Pelt, G, N; Cape May Courthouse, Cape May Co., Long, S; Delair, Camden Co., Long, S; Farmingdale, Monmouth Co., Bayard Long and Stewardson Brown, S; Folsom, Atlantic Co., Long, N, S; Locust Grove, Camden Co., Long, S; Millville, Cumberland Co., Long, S; New Egypt, Ocean Co., J. H. Grove, S; Phillipsburg, U; Somerset Co., R. C. Perry, M; Tomlin, Gloucester Co., Long, S; Tomlin, Gloucester Co., C. S. Williamson,

N; Westville, Gloucester Co., Williamson, N; Woodbury, Gloucester Co., Long, S.

NEW MEXICO. *R. Rydbergii*: Kingston, Sierra Co., Metcalfe, M, N; Kingston, Sierra Co., 6600 ft., O. B. Metcalfe, F; Las Vegas, 6400 ft., E. Tuttle, N; Lincoln Co., U; Mogollon Mts., U; Mogollon Mts., 7500 ft., Metcalfe, N; Salado Canyon, Earle, N; Sandia Mts., U; Sandia Mts., 8100 ft., C. C. Ellis, N; Santa Fe Canyon, U; Santa Fe Canyon, J. N. Rose, N; Ute Park, Colfax Co., U; White Mts., U; Windsor's Ranch, Pecos River National Forest, 8400 ft., Standley, N. *R. Toxicodendron*: . . ., A. Fendler, M, G; Florita Mts., Jones, P (observed); Mogollon Mts., Metcalfe, G, M; Mogollon Mts. (7500 ft.), Metcalfe, P; New Mexico, G. Engelman, S; Water Canyon, Magdalena Mts., Socorro Co., C. J. Herrick, F.

NEW YORK. *R. radicans*: Chemung Co., T. F. Lucy, F; Garretson's, S. I. Britton, N; Montauk Point, Suffolk Co., N. Taylor, N; New Dorp, Britton, N; Springfield, Queens Co., E. G. Knight, N. *R. Toxicodendron*: Broome Co., C. F. Millspaugh, F; Chemung Co., Lucy, F; Depauville, Fernald, Wiegand, and Eames, G; Ithaca, U; Ithaca, W. Trelease, M; Ithaca, Cascadilla Creek, E. L. Palmer, G; Ithaca, Renwick Woods, Wiegand, G; Lansing, Wiegand, G; Lisbon, O. P. Phelps, C, G, N; Long Island, Greenport, E. S. Hoar, G; Montezuma, Eames, Wiegand, and Randolph, G; Selkirk, Sand Dunes, Fernald, Wiegand, and Eames, G; Tioga Co., U; Trenton Falls, West Canal Creek, J. V. Haberer, G; Troy, J. Hall, F.

NORTH CAROLINA. *R. Toxicodendron*: Asheville, Huger, N; Raleigh, U; Tryon, Polk Co., Churchill, G; Tryon, C. F. Millspaugh, F; Wilmington, U.

NORTH DAKOTA. *R. radicans*: Grand Forks, C. A. Egebreton, F. *R. Rydbergii*: Butte, Benson Co., Lunell, N; Minnewaukan, Benson Co., Lunell, N. *R. Toxicodendron*: Fort Clark, F. V. Hayden, M.

OHIO. *R. Toxicodendron*: Cincinnati, C. G. Lloyd, C; Cuyahoga River, Akron, U; Oberlin, U.

OKLAHOMA. *R. negundo*: Kay Co., M. White, N. *R. quercifolia*: McAlester, E. J. Palmer, A; McAlester, U. *R. Toxicodendron*: Boss, McCurtain Co., Houghton, G; Clayton, Waugh, M; Commerce, B. F. Bush, A; Knowles, U; Knowles, Beaver Co., Stevens, G; Page, Palmer, A; Sapulpa, Bush, M; Stillwater, U; Tishomingo, U; Tishomingo, Palmer, A; Tishomingo, Johnston Co., Houghton, G; Yukon, Palmer, A.

OREGON. *R. diversiloba*: Azalea Creek, U; Bridal Veil, Multnomah Co., H. H. Smith, F; Coos Bay, U; Forest Grove, Washington Co., Lloyd, N; Grant's Pass, J. G. Jack, A. Rehder, A; Jackson Co., U; Oakland, L. E. Hunt, A; Portland, Drake and Dickson, F; Portland, Howell, N; Portland, Rusby, N; Portland, U; Rocky Butte, Multnomah Co., E. P. Sheldon, F; Salem, J. C. Nelson, S; Umpqua River, Douglas Co., U; Umpqua River, G. Engelmann and C. S. Sargent, A; Willamette River,



Salem, E. Hall, F; Willamette Hills, A. I. Mulford, N. *R. hesperium* Greene: Deschute's River, U. *R. Toxicodendron*: Dalles, Howell, G; Deschute's R., T. Howell, F; Pendleton, Drake and Dickson, F; Pendleton, Henderson, G; Portland, L. F. Henderson, S.

PENNSYLVANIA. *R. radicans*: Harrisburg, Small, N; Pittsburgh, J. A. Shafer, F; Rafinesque, Alleghany Mts., N; Westmoreland Co., P. E. Pierron, F. *R. Toxicodendron*: Allentown, Lehigh Co., H. W. Pretz, S; Conewago, A. A. Heller, G; Conewago, J. K. Small, F; Grenoble, Bucks Co., Bayard Long, S; Harrisburg, U; Harrisburg, Small, F; Lancaster Co., U; Mercersburg, Detwiller, S; Mountville, A. F. Eby, M; Nottingham Township, Chester Co., F. W. Pennell, S; Philadelphia, J. H. Redfield, M; Wawa, Pennell, S; Perkiomen, Montgomery Co., J. B. Brinton, S; Westmoreland Co., P. E. Pierron, C, F; Wissahickon Ravine, Philadelphia Co., S. S. Van Pelt, S.

RHODE ISLAND. *R. littorales*: Newport, Meams, N. *R. Toxicodendron*: . . . Thurber, G; Providence, U; Wickford, E. F. Williams, G.

SOUTH CAROLINA. *R. quercifolia*: Aiken, T. G. Harbison, A; Aiken, H. Eggert, M; Darlington, A. Rehder, A. *R. radicans*: Anderson, J. Davis, N; Clemson College, House, N. *R. Toxicodendron*: Aiken, Eggert, M; Batesburg, U; Bradley, U; Clemson, House, N; Clemson College, H. D. House, M; Columbia, Richland Co., E. B. Bartram, S; Manning, Clarendon Co., Witmer Stone, S; Oconee Co., U; Paris Mt., Small, N; Pickens Co., U.

SOUTH DAKOTA. *R. Rydbergii*: Black Hills, Miss Pratt, N; Black Hills, National Forest, 5100 ft., J. Murdock, F; Deadwood, W. P. Carr, C, F; Deadwood, Carr, N; Edgemont, Fall River Co., S. S. Visher, F; Hermosa, U; Hermosa, 3500 ft., Rydberg, N; Hillside, U; Little Missouri valley, Harding Co., Visher, F; White River, Shannon Co., Visher, N. *R. Toxicodendron*: . . . , A. G. Johnson, M; Black Hills, U.

TENNESSEE. *R. microcarpa*: Knox Co., Ruth, M. *R. radicans*: Knoxville, Ruth, N. *R. Toxicodendron*: Bolivar, E. J. Palmer, A; Sherwood, H. Eggert, M.

TEXAS. *R. microcarpa*: Belknap, S. Hayes, N; New Braunfels, F. Lindheimer, M. *R. quercifolia*: Bowie Co., E. Eggert, M; Dallas, J. Reverchon, A, M; Denison, Reverchon, M; Hempstead, E. Hall, M; Huntsville, E. J. Palmer, A; Livingston, Palmer, A; Livingston, U; Marshall, Palmer, A; Marshall, U. *R. radicans*: Kerrville, Kerr Co., 1600-2000 ft., A. A. Heller, F; Tarrant Co., A. Ruth, F. *R. rhomboideum* Small: Austin, Palmer, A; D'Hanis, Palmer, A; Granbury, Palmer, A; Kerr Co., Heller, N; Menard, Palmer, A; Montell, Palmer, A; Spanish Pass, Kendall Co., Palmer, A; Tarrant Co., U. *R. Rydbergii*: Tom Green Co., Tweedy, N. *R. Toxicodendron*: Armstrong Co., Palmer, A; Bowie Co., Eggert, M; Columbia, U; Columbia, B. F. Bush, A, M; Crosby Co., U; Dallas, Eggert, M; Dallas, Reverchon, A, G, M; Dallas County, Reverchon, M; Devil's River, Valverde Co., Palmer, A; Floyd Co., U;

Ganado, Palmer, A; Hempstead, E. Hall, F, M, N, P; Houston, Eggert, M; Houston, Lindheimer, G; Kerrville, Heller, M, G; Larissa, Bush, A; New Braunfels, E. Lindheimer, G, M; Richmond, U; San Angelo, Palmer, A; Sutherland Springs, C. S. Sargent, A; Terrell, U; Tom Green Co., U; Willis, R. A. Dixon, F.

UTAH. *R. longipes* Greene: Salt Lake City (City Creek Canyon), F. E. Leonard, N; Springdale, 4000 ft., M. E. Jones, N. *R. Rydbergii*: Beaver Canyon, U; Springdale, U. *R. Toxicodendron*: Cache Mts., Jones, P (observed); City Creek Canyon, 4300 ft., M. E. Jones, F; Farmington, Mrs. J. Clemens, A; Mt. Belknap, Jones, P (observed); Panguitch, Jones, P (observed); Salt Lake City, Jones, P (observed); Salt Lake City, Farmington Canyon, Pammel and Blackwood, G; Springdale, M. E. Jones, M; Springdale (4000 ft.), Jones, P; Uinta Mts., Jones, P (observed); Wahsatch Mts., Watson, G.

VERMONT. *R. radicans*: Barnet, Blanchard, N. *R. Toxicodendron*: Barnet, U; Charlotte, . . . , M; Charlotte, F. H. Hosford, F; Johnson, A. J. Grout, F; Middlebury, E. Brainerd, G; Willoughby Lake, J. R. Churchill, G.

VIRGINIA. *R. quercifolia*: Cape Henry, U; Colonial Beach, U; Princess Anne Co., U. *R. radicans*: Chesapeake Beach Junction, Ruth, N; Marion, Smyth Co., Britton and A. M. Vail, N; Stony Man Mt., Steele, N; Virginia Beach, Britton and Coville, N. *R. Toxicodendron*: Bedford Co., A. H. Curtiss, C, J; Coan, U; Coan, I. Tidstrom, A; Colonial Beach, U; Colonial Beach, Tidstrom, Bartlett, A; Dismal Swamp, U; Georgetown, D. C. (opposite), A. Scott, F; Great Falls of Potomac River, C. S. Williamson, S; Kimball, U; Luray, U; Luray, Steele, G; Marion, Smyth Co., 2100 ft., Britton and Vail, F; Norfolk Co., U; Ocean View, U; Petersburg, A. Rehder, A, M; Virginia Beach, U; Virginia Beach, Princess Anne Co., Britton and J. K. Small, F; Woodlawn, U.

WASHINGTON. *R. diversiloba*: Columbia River, western Klickitat Co., W. N. Suksdorf, F, N; Mercer Island, Seattle, F; Orchard Point, C. V. Piper, A; Yakima Region, T. S. Brandegee, A. *R. hesperium*: Spokane, U; Wartsburg, U; Whitman Co., U. *R. Rydbergii*: Spokane, F. O. Kreeger, N; Wawawai, A. D. T. Elmer, M, N. *R. Toxicodendron*: Hangman Creek, Spokane Co., Suksdorf, G; Klickitat Co., Suksdorf, G; Oroville, Jones, P (observed); Spokane, Jones, P (observed); Spokane, Kreeger, G; Spokane, T. E. Savage, F; Spokane, T. E. Savage, M; Wartsburg, Stream Banks, R. M. Horner, G; Wawawai, Elmer, A, M.

WEST VIRGINIA. *R. radicans*: Morgantown, Monongalia Co., Millsbaugh, N. *R. Toxicodendron*: Hacker Valley, Webster Co., H. H. Smith, ?; Little Falls, C. F. Millsbaugh, F; Upshur Co., U; Upshur Co., W. M. Pollock, M.

WISCONSIN. *R. radicans*: Milwaukee Co., Hasse, N. *R. Toxicodendron*: Elkhart Lake, J. H. Schuette, F; Green Bay, F; Milwaukee, A. Lapham, M; Milwaukee, C. W. Short, S; Milwaukee Co., H. Russel, ?; Richland Center, Richland Co., O. E. Lansing, F.

WYOMING. *R. Rydbergii*: Big Horn, Sheridan Co., Tweedy, N; Evans-ton, L. H. Pammel and R. E. Blackwood, M; Freezeout Hills, E. Nelson, M, N; Hartville, U. *R. Toxicodendron*: Freezeout Hills, E. Nelson, M; Tongue River Canyon, Bighorn Mts., J. G. Jack, A.

#### WEST INDIES

BAHAMAS. *R. Blodgettii*: Andros, Small and Carter, N; Boaz Island, Stewardson Brown and N. L. Britton, N, S; De la Port, New Providence, E. G. Britton, F, N; Eight Mile Rocks, Great Bahamas, Britton and Millspaugh, N; Great Bahama, West End, L. J. K. Brace, F, N; Marsh Harbor, Brace, N; New Providence, Brace, N; North Cat Cay, Millspaugh, N; Paget Marsh, S. Brown and Britton, N, S; Savannah, north section, Andros, J. K. Small, F.

BERMUDA. *R. Floridana* Mearns: Tucker's Town, S. Brown, N, S.

The following is a list of locations at which birds which had eaten fruit of *R. Toxicodendron* were collected:

ALABAMA: Auburn, Autaugaville, Castleberry, Greensboro, Huntsville, Jackson, Scottsboro.

ARKANSAS: Big Lake, Dewitt, Menesha, Mud Lake, Turrell.

BRITISH COLUMBIA: Okanagan Landing.

CALIFORNIA: Alhambra, Hayward, Pasadena, Watsonville.

CONNECTICUT: Bridgeport, Cheshire, East Hartford, East Norwalk, Easton, Fairfield, Meriden, New Haven, Norwalk, Portland, Stamford, Wallingford.

DISTRICT OF COLUMBIA: High Island, Washington.

FLORIDA: Amelia Island, Eau Gallie, Fort Drum, Kissimmee, Melbourne, Micco, Sunbeam, Upper St. Johns River, Walton Co.

GEORGIA: Calhoun.

ILLINOIS: Addison, Aurora, Bloomington, Cairo, Evanston, Galesburg, Grand Tower, Homer, Hudson, Jacksonville, Mound City, Petersburg, Salt Fork, Sangamon River, Springfield, Spring Lake, South Chicago, Twin Grove.

IOWA: Sabula.

KANSAS: Crawford County, Onaga, Zarah.

KENTUCKY: East Cairo, Fillmore, Union Co.

LOUISIANA: Alexandria, Avoyelles Parish, Belcher, Bordelonville, Clarks, Foster, Hamburg, Lecompte, Logansport, Mansura, Natchitoches, Redfish.

MAINE: Scarborough.

MARYLAND: Aikin, Beauveau, Branchville, Chevy Chase, College Park, Laurel, Marshall Hall, Montgomery Co., Silver Springs, Takoma Park.

MASSACHUSETTS: Boston, Lunenburg, Mansfield, Saugus, Weston.

MICHIGAN: Greenfield Township, Palmer, Redford, Richmond, Wayne Co.

MINNESOTA: St. Vincent, Roseau River at U. S. Boundary, Kittson Co.

MISSISSIPPI: Adams Station, Burnsville, Carlisle, Fayette, Flora, Florence, Magee, Michigan City, Morton, Pickens, Raymond, Rolling Fork, Utica, Verna, Vicksburg.

MISSOURI: Little River.

MONTANA: Tityous Ranch, 26 miles from Ft. Buford, in Yellowstone.

NEBRASKA: Badger, Lincoln.

NEW HAMPSHIRE: Nottingham.

NEW JERSEY: Afton, Audubon, Englewood, Fort Lee, Freehold, Hackensack, Morris-town, Overpeck Creek, Palisades Park, Schraalenburg, Shiloh, Stag Lake.

NEW YORK: Astoria, Flushing, Glen Cove, Jamaica, Lawrence, Locust Valley, Long Island City, New York, Rockaway Beach, Rye, Shelter Island, Sing Sing, Suffolk, Syracuse, Van Cortlandt.

NORTH CAROLINA: Currituck, Raleigh, Swan Island, Winston-Salem.

OHIO: Columbus, Oxford, Rockport, Sugar Grove.

OKLAHOMA: Guthrie, Otoe.

ONTARIO: Belle River, Colehuta Township, Hyde Park, Point Pelee, Sandwich, Windsor.

PENNSYLVANIA: Beaver, Chester Co., Ercildoun, Montgomery Co., Tredyffrin Township, Vanport, West Chester, West Philadelphia.

RHODE ISLAND: Providence.

SOUTH DAKOTA: Pierre.

TENNESSEE: Chickamauga.

TEXAS: Columbus, Dallas and Dallas Co., Hempstead, Lake Charlotte, Liberty, Navasota, Old River, Victoria, Winfree.

VIRGINIA: Alexandria, Hampstead, Mason's Neck, Mecklenburg Co., Mt. Vernon, Northumberland Co.

WASHINGTON: Garfield Co., Snake River.

WEST VIRGINIA: Weston, Wheeling.

WISCONSIN: Beaver Dam.

WYOMING: Newcastle.

## A NEW CONJUGATE FROM WOODS HOLE

I. F. LEWIS

(Received for publication September 15, 1924)

A member of the Conjugales found for several seasons at Woods Hole, Mass., offers such distinctive peculiarities of reproduction that it may best be considered as the type of a new genus closely related to *Spirogyra*, and perhaps even more closely to *Choaspis* (*Sirogonium* Kuetzing, 10).<sup>1</sup>

The alga was first found in 1917 in a small pond in the Gansett Woods near Buzzard's Bay commonly known among visiting botanists as Wood Pond. Conditions in the pond vary considerably with the excess or deficiency of the rainfall. In dry summers the water may disappear completely, although usually there is a depth of two feet or even more. Sticks and leaves from the shrubby vegetation in and around the pond accumulate on the bottom along with the remains of grasses and sedges. There is always a rich flora of desmids and Protococcales, though the Myxophyceae are notably scarce. From 1922 to 1924 large quantities of *Nitella* were present.

The alga, for which the name *Temnogyra Collinsii* is proposed, occurs floating or attached to sticks and leaves near the surface of the water. It has not been found in pure growth, being associated and tangled with other filamentous Conjugales such as *Desmidium Schwartzii* and species of *Spirogyra*.

### VEGETATIVE CHARACTERS

The unbranched filaments would be taken at first glance for those of *Spirogyra*, which they resemble except for the lack of a copious mucous sheath. The chloroplast is usually single and quite pale, making 5-7

<sup>1</sup> Gray's description of *Choaspis* (7) antedates Kuetzing's by 22 years. Gray's diagnosis (p. 299) is as follows: "CHOASPIS. *Thallus* threadlike, simple, tubular, jointed, knee-bent; coupling at the bend, by a perforation in each joint, which transmits the granules from one plant to the other, where they form an elliptic spore; *granules* scattered.—Not slippery."

Kuetzing's description, as given by Rabenhorst (13, p. 256) is: "SIROGONIUM Ktz. (1843): Cellulae vegetativae cylindricae, sporiferae subinflatae orculiformes. Fasciae chlorophyllosae longitudinales, parietales, leviter flexuosae, nodosae (plerumque 2-3, rarius 4 in quaque cellula), granula amylacea 7-8 involutae. Copulatio genuflexa, sine tubo connexivo."

It was De Bary (5) who first described the unequal divisions leading to gamete-formation, and it is these which more than anything else lead to the acceptance of *Choaspis* as an independent genus. In relatively modern works there is a tendency to regard it as a subgenus of *Spirogyra*, as was done by De Toni, Collins, and Pascher, while West and Oltmanns accept the genus as independent.

turns in clockwise fashion, and containing 15-20 pyrenoids. The edge of the chloroplast is irregularly toothed and lobed. The nucleus is central, but is not suspended by cytoplasmic strands, as in many species of *Spirogyra*. The cross walls are plane.

At times the chloroplast becomes double by a process of kinesis (Pl. XXXVII, figs. 15-23). It breaks into two parts near one end of the cell, the shorter part consisting of about one turn of the chloroplast. The two fragments draw apart a little and then extend gradually past each other. The extension appears to be due to stretching, as the parts of the chloroplast undergoing it become noticeably tenuous. As the extension proceeds, both portions gradually lose their spiral position and straighten out until they lie side by side in the cell. The shorter one increases more rapidly in length until both extend nearly from one end of the cell to the other, when they begin to coil loosely around each other. Finally the regular spiral is re-established and the typical cell with its single chloroplast has given place to the less usual form of cell with two chloroplasts.

Variation in the number of chloroplasts in *Spirogyra* has been noted by Petit (12) in *S. Grevilleana*, by Andrews (1) in *S. elongata*, and by Kasanowsky (9) in *S. Nawaschini*. In the last-named species the transition from one chloroplast to two is brought about when the end of the chloroplast near the cross wall bends back and grows in the opposite direction. This free end of the chloroplast continues to extend backward until the cell gives the appearance of having two bands. A break at the point of bending makes the appearance a reality. The origin of a second spiral in *Spirogyra* has also been observed by Hill (8a). A similar process has been observed by me in an unidentified (sterile) species of *Spirogyra* from Weeks's Pond east of Pocasset, Massachusetts. The number of chloroplasts also varies in *Choaspis stictica* (*Sirogonium sticticum* Kg.). It is given as 3 by Gray, 2-4 by Kuetzing, and 5-6 by De Bary.

The filaments are sometimes attached by holdfasts which are lateral outgrowths of a vegetative cell. The holdfasts are in length nearly twice the diameter of the cell. They are usually not attached to the support by the mushroom-like tips characteristic of the holdfasts of many Chlorophyceae, but curve like a hook around the support. Holdfasts are described for *Spirogyra* by Delf (6) and others.

#### REPRODUCTION

No form of vegetative multiplication or asexual reproduction has been observed. Sexual reproduction, on the other hand, may be seen at any time during the growing season. At some times it is more abundant than at others, but it never becomes so general as to involve all or nearly all the filaments, as is frequently the case in *Spirogyra*.

Conjugation is either lateral or scalariform. There is no trace of unisexuality in the filaments. Sex resides in the individual cells, and male

and female cells are scattered with no observable regularity throughout the filaments (figs. 24-28). Cross conjugation of the sort illustrated is rare in *Spirogyra*, where as a rule conjugation is either strictly lateral or strictly scalariform, but it has been recorded rather recently by Cunningham (3, 4), who reports two cases (*S. inflata* and *S. Weberi*) and gives in his first paper an excellent historical summary. The subject is discussed at length by Hemleben (8). De Bary (5, p. 15) notes that in *Choaspis stictica* both male and female cells occur in the same filament, though lateral conjugation appears to be absent in this species.

In *Temnogyra* lateral conjugation is more usual than scalariform, though both are common. The first visible sign of approaching conjugation (Pl. XXXVI, fig. 1) is the formation of papillae on neighboring cells quite near the cross wall. One papilla forms a trifle earlier than the other, and is larger. This is destined to carry the male gamete. It grows toward the papilla of the female cell and pushes it inward until at the time of fusion the convex tip of the male papilla occupies a corresponding concavity in the papilla from the female.

During the development of the papillae into the conjugating tube, the filament bends at the point of conjunction of the sexual cells. The angle so formed may be as much as 60 degrees, but varies between this and almost zero.

While the conjugating tube is forming, the chloroplast, first that of the male cell and then that of the female, may be seen to be crowding into the end of the cell, and the nucleus moves in the same direction. The displacement of the chloroplast is obvious in the neighborhood of the nucleus (fig. 2, right), and suggests the contraction of the chloroplasts produced by barium (Chien, 2), which causes the coils of the chloroplasts to crowd together around the nucleus. Such crowding is relieved in *Temnogyra* by the slipping of the chloroplast toward the conjugation papilla and by the movement of the nucleus. Before the chloroplast slips entirely past the nucleus the latter divides. The cross wall whose formation follows nuclear division divides the originally long cell into two, one short with a nucleus and the greater part of the chloroplast, the other long with a fragment of the chloroplast and a nucleus in contact with it (figs. 3-6). The shorter cell contains the gamete, the longer is the sterile cell.

The male gamete is invariably cut off sooner than the female (figs. 3, 4). It does not increase notably in diameter, as does the female gamete, and is further distinguished by the fact that the chloroplast disintegrates completely, losing both color and form. Disintegration of the chloroplast begins in the portion nearest the conjugating tube, and progresses until the whole chloroplast is involved (figs. 5, 6). There is no visible plasmolysis during these changes, either in the gametes or in the sterile cells. Attempts to demonstrate by the method of plasmolysis a difference in osmotic pressure between the cells taking part in the conjugation process and normal vegetative cells gave negative results.

The mature male gamete consists of a granular mass of protoplasm, in which lie the pyrenoids from the disintegrated chloroplast and a single nucleus. Just before fusion a vacuole appears in that portion of the male gamete occupying the conjugation tube. The female cell is considerably swollen, but the chloroplast and the usual cell organization are retained intact.

The protoplast of the male gamete passes into the female cell cavity and there fuses with the female protoplast. The resulting zygospore (figs. 9, 12, 14) is oval, with a straw-colored scrobiculate mesospore and a thinner smooth endospore and exospore. In its protoplast, dense with oil and starch, may sometimes be seen the much shortened and thickened chloroplast from the female gamete.

Germination has been observed only on one occasion. The material exhibiting the phenomenon was collected in late summer, allowed to dry slowly, and again moistened in the spring. The first sign of beginning germination is the development of a green color in the chloroplast, which thus becomes more conspicuous. Further progress of germination resembles that in *Spirogyra* or *Zygnema* (fig. 13).

In scalariform conjugation, the march of events is similar to that in lateral conjugation (figs. 7-12). Cases have been observed in which the gamete protoplasts failed to fuse after the male protoplast had passed into the female cell cavity. In such cases both gamete protoplasts round off independently, the larger female becoming oval, the smaller male spherical (fig. 10). Inspection of the figures will show that the considerable difference in size between male and female gametes is not associated with a size difference in the filaments. The increase in size of the female gamete occurs at about the time the pre-fusion vacuole is seen in the male gamete. The genuflexion so characteristic of lateral conjugation (figs. 3, 5, 6) is usually absent in scalariform conjugation (figs. 7, 8), though some trace of it is not rare (fig. 11, female).

In scalariform conjugation there is considerable variation in the degree of development of the portion of the conjugation tube contributed by the female cell. Sometimes it is nearly as well developed as that from the male cell (fig. 7). Again it is hardly developed at all (fig. 11). Intermediate stages between these extremes are common. Very often the point of contact between the two parts of the conjugation tube is plane; the convex bulge of the male portion is often absent, which is not the case in healthy lateral conjugation.

#### COMPARISON WITH SPIROGYRA AND CHOASPIS

*Temnogyra* differs from *Spirogyra* in one decisive respect and in several minor details. The feature of its life history which makes it impossible to unite this form with the older genus without an amended definition of the latter is the production of specialized gametes formed by the unequal



division of a mother cell. Other features which either do not occur in *Spirogyra* or are rare in that genus are:

1. The bending of the filaments at the point of copulation, like that seen in *Mougeotia*.
2. The presence of hook-shaped holdfasts occurring laterally on the cells.
3. The swelling of the female gamete, resulting in a marked contrast in size between male and female.
4. The indiscriminate way in which lateral, cross, and scalariform conjugation occur.
5. The absence of a mucous sheath.

The relation of *Temnogyra* to *Choaspis* (*Sirogonium*) is not so clearly defined. Certain points are very similar in both, such as the swollen female gametes, the absence of mucus, and the fact that conjugation may take place at practically any time during the growing season, rather than be restricted to a certain definite season as was established for *Spirogyra* by the careful work of Transeau (14). The differences, however, are striking.

<i>Temnogyra</i>	<i>Choaspis</i>
Chloroplast a regular spiral.	Chloroplasts nearly straight, parallel.
Conjugation tube well developed.	Conjugation tube absent.
Cell division leading to gamete-formation differential, the gamete receiving the bulk of the chloroplast, the sterile cell relatively empty.	Cell division leading to gamete-formation not differential, the sterile cells resembling vegetative cells in structure.
Gamete always smaller than the single sterile cell.	Gamete smaller or larger than the one or two sterile cells.
Genuflexion in lateral conjugation.	Genuflexion in scalariform conjugation; lateral absent.

The first three differences mentioned above are of generic order, an opinion which was shared by the late Frank S. Collins, whose highly appreciated advice and criticism were freely offered. The relation of *Temnogyra* to *Spirogyra* is almost identically that of *Temnogametum* (15, p. 37) to *Mougeotia*. In some respects *Temnogyra Collinsii* shows a resemblance to *Spirogyra punctiformis* Transeau, and in this species approaches most nearly the genus *Spirogyra*.

***Temnogyra* gen. nov.** Vegetative characters of *Spirogyra*, chloroplast spiral, cross walls plane; conjugation lateral, scalariform, or cross, beginning in vegetative cells from which smaller gametes are cut off after one nuclear division; bulk of chloroplast passing into gamete; conjugation tube present; female gametangium swollen, containing zygospore.

***T. Collinsii* sp. nov.** Chloroplast single, rarely double, making about five turns; vegetative cells 18–22 x 125–250  $\mu$ , male gametangia 18–22 x 27–54  $\mu$ , female 25–35 x 45–65  $\mu$ ; zygospore oval, brown, with scrobiculate mesospore, 30–35 x 52–62  $\mu$ .

Hab. Wood Pond, Weeks's Pond, Barnstable County, Massachusetts.

Type specimen deposited in the Farlow Herbarium of Harvard University.

#### DISCUSSION OF SEXUALITY

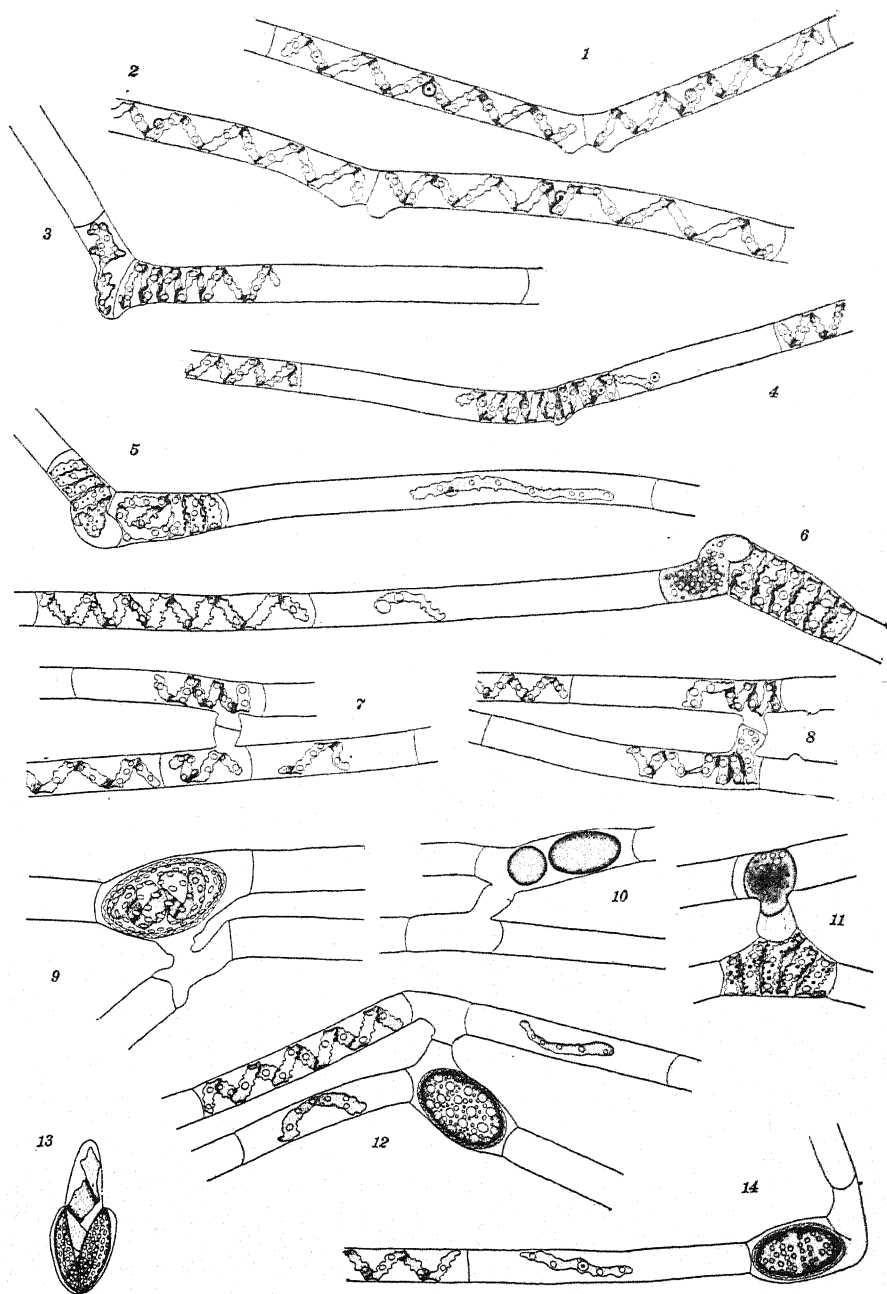
According to the usual conception of chromosome reduction in the Zygnemaceae (see Oltmanns, 11, pp. 103-104) this occurs during the first divisions of the fusion nucleus. Four nuclei are produced in the process, of which three degenerate. The observations on which this account is based were all made on unisexual (scalariform) species. Leaning too heavily on zoological analogies, though with some confirmatory evidence, especially from Sphaerocarpos, botanists seem inclined to suppose that the determination of sex is associated with or may be traced back to the reduction divisions. This can hardly be true, however, where the separation of the sexes occurs in a monoecious sporophyte (as in most seed plants) or in a monoecious gametophyte (as in many archegoniates). If found at all, it must be looked for in forms with a dioecious haploid generation, as in some archegoniates and many algae.

In *Temnogyra* there are two possibilities, mutually exclusive. Either reduction does not occur in zygosporo-germination, or sex-determination is not associated with chromosome reduction. The complete way in which male and female cells may be found scattered at random along the filaments is shown in figures 26-28, Plate XXXVII. Speculation is hardly profitable until chromosome reduction has been worked out for forms with lateral and especially with cross conjugation.

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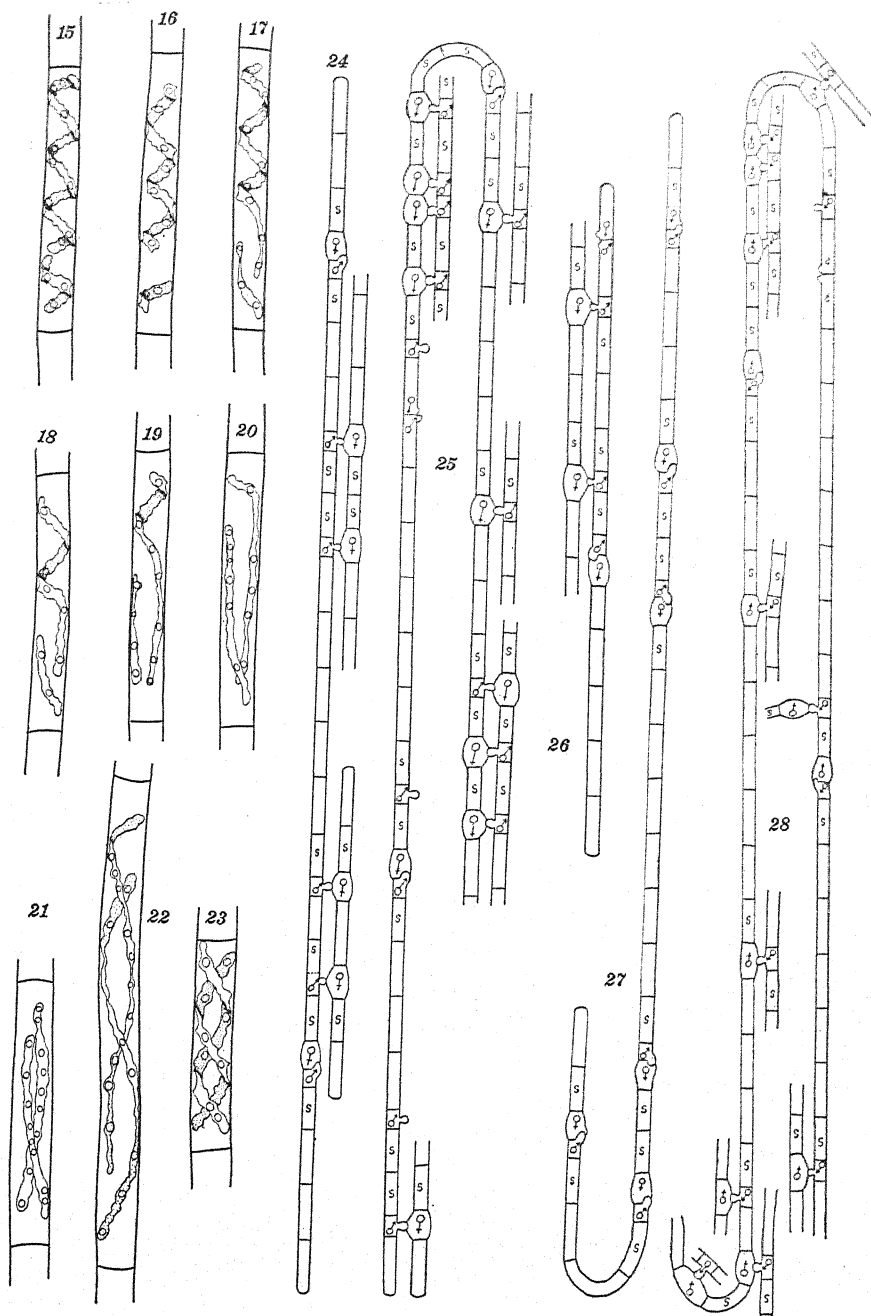
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LEWIS: A NEW CONJUGATE





LEWIS: A NEW CONJUGATE



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## EXPLANATION OF PLATES

Figs. 1-8, 10-14, 15-23,  $\times 244$ . Fig. 9,  $\times 288$ . Figs. 24-28,  $\times 92$ . All drawings from life; in figures 1, 2, and 4 the nuclei were inserted after the addition of iodine in potassium iodid.

## PLATE XXXVI

FIG. 1. The beginning of lateral conjugation. In the cell on the right the nucleus and chloroplast have begun to move toward the papilla.

FIG. 2. Same, slightly later; movement of nucleus and chloroplast more pronounced.

FIG. 3. Pronounced genuflexion; male gamete going to pieces, the movement of material about complete in female cell.

FIG. 4. Male cell (on right) divided into gametangium and sterile cell; female nucleus not yet divided.

FIG. 5. Both gametangia cut off; male shows beginning of degeneration of chloroplast; sterile cell accompanying female shows fragment of chloroplast and nucleus.

FIG. 6. Older stage; chloroplast of male gamete disorganized; pre-fusion vacuole visible; female gametangium swollen; sterile cell and adjoining vegetative cell shown.

FIG. 7. Scleriform conjugation; male gametangium cut off from its sterile cell; division just beginning in female.

FIG. 8. Slightly earlier stage; male chloroplast beginning to degenerate before cell division is completed; female cell (above) not yet divided; note connecting tube and absence of genuflexion.

FIG. 9. Mature zygospore in female gametangium; much thickened female chloroplast visible.

FIG. 10. Male gamete has passed into female cell; fusion has failed to take place.

FIG. 11. Fusion about to begin; note pre-fusion vacuole; female filament shows slight genuflexion.

FIG. 12. Mature zygospore; two sterile cells visible; apparent genuflexion probably due to pressure of cover glass.

FIG. 13. Germinating zygospore.

FIG. 14. Zygospore of lateral conjugation.

## PLATE XXXVII

FIGS. 15-23. Successive stages in transition from single to double chloroplast.

FIGS. 24-28. Diagrams of filaments illustrating distribution of sexual cells, occurrence of lateral and cross conjugation in the same filaments, and position of sterile cells (s).





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## ACIDITY AND VARIETAL RESISTANCE OF WHEAT TO *TILLETIA TRITICI*

ANNIE MAY HURD-KARRER

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In former papers it was shown that varietal resistance of wheat to stem rust (*Puccinia graminis tritici*) is not correlated with either the titratable-acid (29) or the hydrogen-ion concentration (28) of the expressed juice of the plants. However, with regard to infection by bunt (*Tilletia tritici*), Kirchner (32; 33, p. 108) reports that susceptible wheat seedlings are less acid than resistant plants, and suggests that high titratable-acid concentration is responsible for resistance. It seemed desirable, therefore, to make a more exhaustive study of both the hydrogen-ion concentration and the titratable acidity of wheat varieties whose reactions to the smut organism range from extreme susceptibility to immunity. The data obtained in this study are recorded in the following pages.

The literature bearing on the question of a relation between cell-sap acidity and disease resistance indicates a widespread belief that these are related characters, but leaves the impression that the data on which positive claims are based are too often meager or unconvincing.

### • REVIEW OF LITERATURE

Comes (12-15) is credited with having been the first to claim that varietal resistance or susceptibility to certain plant and animal parasites can be explained on the basis of differences in cell-sap acidity. He came to this conclusion through his work on resistant varieties of grapes, citrus fruits, and cereals, his Rieti wheat being the conspicuous example of resistance to rust conferred, supposedly, by high acid content. The same explanation was given by Averna-Saccà (4-6) for the resistance of grapevines to Phylloxera, Peronospora, and Oidium. Both he and Comes emphasized the fact that cultivation, manuring, liming, transplanting to warmer climates, or any treatment which results in decreased cell-sap acidity, decreases resistance. Confirmatory results were reported by Degli Atti (18, 19), who studied grape varieties resistant to Phylloxera; and by

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Campbell (10), who increased the resistance of cultivated trees susceptible to various parasitic organisms by causing them to absorb acids and also by grafting on them scions taken from resistant wild trees with more acid sap.

Kirchner (32, 33) found more titratable acid in two rust-resistant varieties and in one smut-resistant variety, than in any of an equal number of susceptible varieties. Appel (2) stated that the presence of benzoic acid is supposed to be the cause of the resistance of *Vaccinium vitis-idaea* to fungous diseases. Hiltner (26) found that Robinia plants were immune from, or highly susceptible to, mildew according to the acidity or alkalinity of the nutrient solution. Laurent (34) increased the resistance of potato tubers to bacterial rot by causing the tissues of the tuber to absorb acid solutions. Consistent with these results was his claim that the application of lime to the soil decreases resistance, while phosphoric acid increases it. However, when he titrated the expressed juice of untreated tubers of resistant and of susceptible varieties, he found no relation between cell-sap acidity and resistance. Arrhenius (3) made the statement that if we were able to increase the "potential acidity" of the cell sap, we should have a means of regulating the disease susceptibility of the plant.

Recently Marañón (36) has reported a conspicuously greater total acid content in the leaves of strains of *Oenothera* which are resistant to powdery mildew, as compared to the corresponding values for leaves of susceptible strains. However, equally distinct correlations with resistance were found in the measurements of other chemical constituents. So Marañón concluded that resistance is associated with a factor complex which is inherited as a whole, and which is not disintegrated by hybridization. He makes the statement: "Factors that increase tannin and acid and that decrease water-soluble nitrogenous compounds tend to build up immunity."

Many investigations which have indicated a sensitiveness of bacteria and fungi to the hydrogen ion (21, 22, 37, 55), or have established the toxicity of acid substrates for disease-producing organisms (8, 16, 20), have helped confirm the widespread suspicion that cell-sap acidity may be a determining factor in the ability of the host plant to resist invading organisms.

Investigators are not all agreed, however, that high acidity bears a causal relation to immunity. Carlucci (11), Grimaldi (23), Pantanelli (39), and Sannino (48, 49, 50) expressed doubts, the latter noting that *Peronospora* and *Oidium* attacked virulently those vines that were highest in acid content. Viala and Pacottet (54) made the observation that "black rot" of vines attacked virulently tissues that were relatively rich in acids. Petri (43, 44) refused to accept the idea that cell-sap acidity can be taken as a criterion of the ability of grape varieties to resist *Phylloxera*. A little later, Lo Priore (35) repeated the observation that high acidity did not prevent virulent attacks of *Peronospora* and *Oidium*. Paris (40, 41, 42) also denied the validity of Comes' theory and the work of subsequent investigators purporting to prove it, and in support of his refutation gave data obtained from

his analyses of the roots, stems, and leaves of various grape varieties, some resistant and some susceptible to *Phylloxera* and *Oidium*.

Rivera (45) thought that cell-sap acidity is not the factor determining the resistance of wheat to *Erysiphe graminis*. Vavilov (53) showed that resistance of oats to mildew, rust, and loose smut, and of wheat to mildew and rust, is independent of the acidity of the leaves. Nevertheless he claimed to have found a direct correlation between the acidity of the seeds and the relative susceptibility of the plants. Scurti and Sica (52) concluded that Comes' theory, properly interpreted, has a high degree of probability; yet they were unable to obtain the exact correlation between acidity and rust resistance demanded by the theory. Henning (25) concluded from Bygdén's (9) analyses that the titratable acidity of wheat varieties bears no relation to their respective susceptibilities to yellow rust. Likewise, the hydrogen-ion measurements made by Spriesterbach<sup>1</sup> and Arrhenius (2b) on wheat varieties varying in degree of resistance to rust, and by Arland (2a) on oat varieties variously susceptible to smut, show no correlation between resistance and cell-sap acidity.

Walker (56) found that the variation in the acidity of extracts of scales from onion bulbs was insufficient to account for the resistance of some and the susceptibility of others to *Colletotrichum circinans*. Measurements on the juice of potato tubers have not shown any relation between acidity and disease resistance. Thus Appel (1) concluded that resistance to bacterial tuber rot is unrelated to the acidity of the cell sap; Jones, Giddings, and Lutman (31), that there is no correlation of these characters among potatoes variously susceptible to *Phytophthora infestans*; Hawkins and Harvey (24), Weiss and Harvey (58), and Moore (38), that the hydrogen-ion concentration of the juice of the tubers is not responsible for the ability of the variety to resist the attacks of *Pythium debaryanum*, of *Chrysophlyctis endobiotica*, or of *Fusarium coeruleum*, respectively.

Mention should be made also of the work that has been done on the relation of acidity to the susceptibility of fruits to molds and rot-producing organisms. Zschokke (60) claimed that the resistance of certain apples and pears to various fungi which produce fruit rots is due to higher tannin and malic-acid content. Behrens (7), however, could not subscribe to this opinion, his own observations showing that high acid content plays no rôle in determining the degree of susceptibility of fruits to rots. In particular, he found that a high malic-acid content produces no unfavorable effect on the growth of *Penicillium* in cultures, and does not protect against its entrance into fruits. Cooley (17) found no causal relationship between low acidity of plum fruits and susceptibility to *Sclerotinia cinerea*, since, as the fruit matures, its acidity as well as its susceptibility increases. Willaman and Sandstrom (59) reported that the juice of plums of varieties

<sup>1</sup> Unpublished report, referred to by Gortner in Minn. Agr. Exp. Stat. Ann. Rep. 26: 40. 1917-1918.

resistant to *S. cinerea* has a slightly greater hydrogen-ion concentration and a lower titratable acidity than the juice of susceptible fruits. However, the paper does not contain enough positive data to make this statement convincing, and the authors themselves do not stress the importance of the small differences in the values they obtained.

That the hydrogen-ion concentration may exercise a determining influence on infection in a different sense has been suggested recently by Robbins (46, 47). He mentions the possibility that acidity may bear some relation to disease resistance by virtue of its effect on water-absorption or on other functions which reach a minimum at the isoelectric point of the cell proteins. This theory might also find some support in such data as those obtained by Webb (57), Hursh (30), Scott (51), Hopkins (27), and others.

### METHODS

Investigation of the problem of resistance to *Tilletia tritici* is simplified by the fact that the period of infection is limited to the early seedling stage. The most susceptible period has been shown by many investigators to extend from the breaking of the seed coat to the emergence of the leaves from the coleoptile, during which time the seedling is subsisting almost wholly on the endosperm of the seed. Since the causes of resistance are therefore operative while the plant is independent of external food sources, it is possible to use seedlings grown in germinators as material for physiological studies. Such material possesses distinct advantages in that the plants are uncontaminated by soil or dust, and in that varietal differences in acidity, which might be easily obscured by variability in the external conditions necessarily accompanying photosynthetic activity and soil contacts, would be more apparent in germinator seedlings absorbing only the food reserves of the seed.

Etiolated plants were grown to a height of about three inches on clean cotton cloths or blotters in unlighted germination chambers equipped with thermoregulators. They grew vigorously under these conditions, with no external source of food except the tap water with which the cloths and blotters were saturated. Two resistant and two susceptible varieties were always grown in each germinator, on trays placed one above the other. The relative positions of the trays were varied in different germinators to compensate for any non-uniformity of conditions within the chambers. The plants were cut off just above the seed, seven or eight days after sowing.

The sap was expressed and the acidity measurements were made as rapidly as possible, according to a uniform procedure. The details of the methods used are given in an earlier paper (28).

Determination of the hydrogen-ion concentration of each sample was followed by an electrometric titration. The number of cubic centimeters of N/20 NaOH required to change the hydrogen-ion concentration of a 10-cc.

sample of juice to the value represented by pH 8.3 was taken to represent the "titratable acidity" of the sample.<sup>2</sup>

### RESULTS

The data in table I were obtained from seedlings grown in germinators at a temperature of approximately 20° C., from seed sent from Pullman, Washington, by Dr. E. F. Gaines. Unfortunately, so little seed of Martin was available that but two measurements could be made on this variety. With the exception of White Odessa, which always grew more rapidly than the other varieties, most of the young plants had not broken through the coleoptile at the age of seven days when they were cut. Each pH value given in the table is an average of three successive measurements on the same sample.

TABLE I. *Acidity of 7-day-old Germinator Seedlings of Wheat Varieties Resistant (R) and Susceptible (S) to Tilletia tritici*

Date of Determination 1921	R Hussar C. I. 4843	R White Odessa C. I. 4651	R Martin C. I. 4463	S Jones Fife C. I. 4468	S Hybrid 128 C. I. 4229	S Jenkin C. I. 5177
Hydrogen-ion Concentration Expressed as pH						
Nov. 8.....	6.06	6.01	6.03	....	6.05	6.02
Nov. 22.....	6.05	5.94	6.07	6.03	5.99	5.97
Nov. 23.....	6.05	6.03	....	6.04	....	5.99
Nov. 25.....	6.01	6.03	....	....	5.94	5.97
Dec. 9.....	5.99	6.01	....	5.95	....	5.98
Dec. 10.....	6.00	5.91	....	6.02	....	5.97
Average.....	6.03	5.99	6.05	6.01	5.99	5.98
Titratable Acid Expressed as cc. N/20 NaOH						
Nov. 8.....	6.3	6.1	5.4	....	5.7	4.9
Nov. 22.....	6.8	6.4	5.1	5.4	5.5	4.9
Nov. 23.....	6.6	6.5	....	6.2	....	....
Nov. 25.....	6.5	5.8	....	....	5.8	4.9
Dec. 9.....	6.7	5.9	....	5.8	....	5.0
Dec. 10.....	6.3	6.3	....	6.1	....	4.9
Average.....	6.5	6.2	5.3	5.9	5.7	4.9

There are no consistent differences in the hydrogen-ion concentrations of the different varieties in this experiment. There are, however, some marked differences in the titratable-acid values. Hussar, an immune

<sup>2</sup> pH 8.3 was chosen in order to permit direct comparisons with the results of colorimetric titrations for which phenolphthalein is commonly used. Since the volume of alkali required to reach this or any other end-point is determined by the buffer substances present as well as by the acids, the term "titratable acidity" rather than "total acidity" is used throughout this paper.

variety, has the highest titratable acidity, while Jenkin, a very susceptible variety, has the lowest. These relations appear not only in the averages but in each day's results considered independently of the rest. White Odessa, which is very resistant, is but little less acid than Hussar. The values for the susceptible Hybrid 128 and Jones Fife are, for the most part, intermediate. The relatively low acidity of Martin, an immune variety, contradicts the suggested correlation of acidity with resistance.

The data in table 2 were obtained from plants grown from seed sent by Mr. H. M. Woolman from Corvallis, Oregon. The small quantity of seed available made it impossible to duplicate the determinations. Temperatures in the germinators were approximately 20° C. throughout.

TABLE 2. *Acidity of 8-day-old Germinator Seedlings of Wheat Varieties Resistant and Susceptible to Tilletia tritici*

Variety	C. I. No.	pH	Titratable Acid (Expressed as cc. N/20 NaOH)
Resistant			
Hussar.....	4843	5.99	5.8
Martin.....	4463	6.07	6.1
Banner Berkeley.....	7362	5.96	6.4
Banner Berkeley.....	7362	5.98	6.3
Turkey (Wash. 326).....	6175	5.94	6.0
Susceptible			
Jones Fife.....	4468	5.95	6.0
White Winter.....	5220	5.81	5.3
Hybrid 128.....	4229	5.88	5.3
Jenkin.....	5177	5.80	5.2

In this series of measurements, three susceptible varieties, White Winter, Hybrid 128, and Jenkin, have the highest hydrogen-ion concentrations and the lowest titratable-acid values. The relatively high titratable-acid values of Jones Fife and Martin are somewhat anomalous, since in other experiments the titratable acidity of these varieties is low.

In 1922, Dr. E. F. Gaines sent from Pullman a second selection of seed of the same strains as those listed in table 1, with the addition of Redit, C. I. 6703, Florence, C. I. 4740, and Little Club, C. I. 4066. All the data given in the tables which follow were obtained from seedlings grown from portions of these seed lots.

The first sowings were in germinators maintained at lower temperatures than those of the earlier experiments. The results reported in table 3 are from plants grown at a temperature which fluctuated somewhat around 18° C.

The hydrogen-ion concentrations for the susceptible varieties, Jones Fife, Hybrid 128, and Jenkin, are higher than those of the other varieties, again showing that high hydrogen-ion concentration does not cause resistance. The almost equally high values for Hussar are not in agreement with the relatively low values for this variety in other experiments.

TABLE 3. *Acidity of 7-day-old Germinator Seedlings of Wheat Varieties Resistant (R) and Susceptible (S) to Tilletia tritici*

Date of Determination 1923	R Hussar	R White Odessa	R Ridit	R Flor- ence	R Martin	S Little Club	S Jones Fife	S Hybrid 128	S Jenkin
Hydrogen-ion Concentration Expressed as pH									
Nov. 5.....	5.91	....	6.03	6.03	5.96	5.95	5.93	5.93	5.84
Nov. 6.....	5.89	6.03	6.00	....	5.93	5.96	5.90	5.90	5.85
Nov. 8.....	5.89	6.04	....	6.02	5.93	5.97	5.87	5.88	5.86
Nov. 10.....	....	6.01	6.02	....	5.96	5.98	5.85	5.85	5.89
Nov. 19.....	5.91	6.01	5.98	6.02	....	5.96	5.87	5.91	5.83
Average.....	5.90	6.02	6.01	6.02	5.95	5.96	5.88	5.89	5.85
Titratable Acid Expressed as cc. N/20 NaOH									
Nov. 5.....	....	....	5.8	6.7	5.0	6.0	5.1	4.6	5.0
Nov. 6.....	4.8	4.8	4.8	....	4.3	5.0	4.4	4.2	4.9
Nov. 8.....	5.2	5.0	....	6.4	4.4	5.8	4.9	....	5.2
Nov. 10.....	....	5.4	5.8	....	5.3	5.0	5.2	4.8	5.2
Nov. 19.....	5.3	5.1	4.9	6.4	....	4.9	4.6	4.2	4.9
Average.....	5.1	5.1	5.3	6.5	4.8	5.3	4.8	4.5	5.0

The relatively low titratable-acid values for Martin, which is an immune variety, are so similar to those for the susceptible Jones Fife, Hybrid 128, and Jenkin, that there is obviously no correlation between titratable acidity of the juice and degree of resistance to *Tilletia tritici* in these varieties. Furthermore, the values for Little Club, an extremely susceptible variety, do not agree as closely with those of the other susceptible varieties as they do with those of the very resistant Hussar, White Odessa, and Ridit.

TABLE 4. *Acidity of 8-day-old Germinator Seedlings of Wheat Varieties Resistant (R) and Susceptible (S) to Tilletia tritici*

Date of Determination 1923	R Hussar	R White Odessa	R Ridit	R Martin	S Little Club	S Jones Fife	S Hybrid 128
Hydrogen-ion Concentration Expressed as pH							
Nov. 23.....	6.00	6.03	6.02	6.03	5.97	5.96	5.96
Nov. 24.....	6.09	6.11	6.05	6.13	6.00	5.97	6.02
Nov. 26.....	6.08	6.04	6.02	6.02	5.94	5.95	5.97
Average.....	6.06	6.06	6.03	6.06	5.97	5.96	5.98
Titratable Acid Expressed as cc. N/20 NaOH							
Nov. 23.....	6.2	6.1	5.9	5.2	5.6	5.0	4.6
Nov. 24.....	5.6	6.6	5.6	4.8	5.7	4.6	4.7
Nov. 26.....	6.0	6.9	5.7	5.1	5.8	4.7	4.9
Average.....	5.9	6.5	5.7	5.0	5.7	4.8	4.7

The data in table 4 were obtained with plants grown at a temperature of 22° C. To prevent the development of molds at this higher temperature, the seeds were sterilized with a 1-1000 solution of mercuric chlorid and then thoroughly washed. Germination was less rapid than it had been in the previous sowing, so that the plants were cut after eight days, by which time they had developed to the point reached by the preceding set of plants in seven days. Jenkin and Florence were not included in this experiment on account of a scarcity of seed of these varieties.

The juices of Jones Fife and Hybrid 128 again have relatively high concentrations of hydrogen ions and low concentrations of titratable acid. The relatively low titratable-acid values for the immune Martin and the high values for the susceptible Little Club are also consistent with earlier results.

A final experiment is reported in table 5. The seed of Hussar and Jenkin had been exhausted, and enough of Hybrid 128 and Florence remained for only one and two determinations, respectively. The retardation in the growth of the previous sowing did not appear in this set of plants, probably because the temperatures in the germinators were raised to 25° C. Therefore the plants were cut at the age of seven days instead of eight.

TABLE 5. *Acidity of 7-day-old Germinator Seedlings of Wheat Varieties Resistant (R) and Susceptible (S) to Tilletia tritici*

Date of Determination 1924	R White Odessa	R Ridit	R Florence	R Martin	S Little Club	S Jones Fife	S Hybrid 128
Hydrogen-ion Concentration Expressed as pH							
Jan. 9.....	6.08	5.94	....	5.92	6.08	5.80	....
Jan. 10.....	6.02	6.02	....	6.01	6.06	5.97	5.90
Jan. 11.....	6.07	6.02	6.09	5.98	6.07	5.96	....
Jan. 14.....	6.11	6.03	....	5.91	6.08	5.90	....
Jan. 15.....	5.95	6.03	....	5.92	6.07	5.95	....
Jan. 16.....	6.05	6.03	....	5.99	6.04	....	....
Jan. 17.....	6.10	5.98	6.10	5.97	5.96	6.00	....
Jan. 19.....	6.14	....	....	5.98	5.98	....	....
Average.....	6.07	6.01	6.10	5.96	6.04	5.93	5.90
Titratable Acid Expressed as cc. N/20 NaOH							
Jan. 9.....	6.4	7.3	....	5.8	5.6	....	....
Jan. 10.....	6.1	5.7	....	5.5	5.7	5.0	4.5
Jan. 11.....	5.9	6.7	7.2	5.1	5.7	5.3	....
Jan. 14.....	6.4	6.9	....	5.7	6.2	5.7	....
Jan. 15.....	6.5	7.1	....	5.5	6.3	5.7	....
Jan. 16.....	6.7	7.2	....	5.8	6.2	....	....
Jan. 17.....	6.6	7.4	6.9	5.5	6.5	5.3	....
Jan. 19.....	6.4	....	....	5.6	5.8	....	....
Average.....	6.4	6.9	7.1	5.6	6.0	5.4	4.5



The relatively high hydrogen-ion concentrations in the juice of the susceptible Jones Fife and Hybrid 128 are in agreement with the results of preceding experiments. Also according to precedent are the low titratable-acid values for these varieties and for the immune Martin, as compared with the corresponding measurements for the resistant White Odessa, Redit, and Florence, and for the susceptible Little Club.

#### DISCUSSION

Although the acidity determinations for each variety usually agree well within the same experiments, there is sometimes an appreciable difference between the values for the same variety in different experiments. This variability indicates either an extreme sensitiveness to slightly different growth conditions prevailing in the germinators at different times, or else that differences exist in the vitality or the physiological condition of different seed-lots of the same variety sufficient to influence the acidity of the seedlings. However, there were no visible differences in rate or vigor of growth which were correlated with the acidity differences.

The relatively high hydrogen-ion concentrations which in most of the experiments characterized the susceptible varieties, Hybrid 128, Jenkin, and often Jones Fife also, as compared with the lower values for the resistant varieties, Hussar, White Odessa, Martin, Redit, and Florence, show that susceptibility to *Tilletia tritici* is not correlated with low hydrogen-ion concentration, nor is resistance correlated with high hydrogen-ion concentration, in the expressed juice of wheat.

Four susceptible varieties, Hybrid 128, Jenkin, Jones Fife, and White Winter, were found to have relatively low titratable-acid concentrations, while the resistant varieties, Hussar, White Odessa, Redit, Florence, and possibly Banner Berkeley and Turkey (Washington 326), had higher values. But the measurements for Martin and Little Club refute the generalization suggested by these correlations. Martin is immune, yet its titratable acidity was so low that it would be placed with the susceptible group if classified on the acidity basis. Little Club is extremely susceptible, yet its titratable acidity was relatively high. In every experiment in which they both occurred, the titratable acidity of Little Club was greater than that of Martin.

Thus it appears that the relatively high titratable acidity of Little Club and the low titratable acidity of Martin are, throughout the experiments, the conspicuous contradictions to Kirchner's (32, 33) suggestion, which might otherwise find some support in the data, that titratable acidity and resistance to *Tilletia tritici* are correlated. At least, if low titratable acidity be correlated with susceptibility in Jenkin, Hybrid 128, and Jones Fife, then some other cause must be sought for the susceptibility of Little Club; and if high titratable acidity be responsible for the immunity of Hussar, then some other cause must be sought for immunity in Martin.

## SUMMARY

The wheat varieties studied in this investigation can be classified into two groups on the basis of the relative titratable-acid concentration of their juice. Those in the group characterized by low titratable acidity are Jones Fife, Hybrid 128, Jenkin, Martin, and probably White Winter. Those in the group characterized by high titratable acidity are Hussar, White Odessa, Ridit, Florence, Little Club, and probably Banner Berkeley and Turkey (Washington 326).

The relatively low values for the immune Martin and the high values for the very susceptible Little Club are inconsistent with Kirchner's suggestion that high titratable acidity of the juice of wheat is responsible for resistance to *Tilletia tritici*.

Hybrid 128, Jenkin, and usually Jones Fife, were characterized by relatively high hydrogen-ion concentrations, as was probably one other susceptible variety, White Winter, thus precluding the existence of a causal relation between a high concentration of hydrogen ions in the juice and resistance to *Tilletia tritici*.

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## ROOT-HAIR ELONGATION IN KNOP'S SOLUTION AND IN TAP WATER

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Notwithstanding the considerable amount of research which has been done upon the conditions favorable to or inhibiting root-hair production, and the attention which has been paid to the nature of the walls of root hairs, together with some work, notably that of Kurt Seidel (19), upon their tropistic responses, as yet almost no investigations have been directed toward a study of their rate of elongation under different conditions. The rate of elongation of roots, leaves, and stems has been repeatedly measured, and these measurements are being used as a basis for the modern mathematical formulae for growth. Hyphae and algal cells have been employed to the same end. However, for an intimate study of growth processes root hairs afford certain marked advantages. They permit of observations on a single aspect of growth, namely, cell-enlargement, without its being affected, to any appreciable extent, by the other two factors, cell-division and cell-differentiation. Furthermore, they are individual cells of a multicellular organism, so that their enlargement is a considerably less complicated process than the enlargement of a multicellular tissue, like a stem or root, and at the same time their behavior may be expected to be more akin to that of the typical cells of the higher plants than is the case with the structures of cryptogams. Finally, during elongation the diameter of a root hair remains approximately constant, so that the increase in length is proportional to the increase in volume. Their volume changes can thus be far more accurately determined than is the case with the prismatic tissue cells, with which Ursprung and Blum (23) have been recently working.

Almost the only work involving the determination of the rate of growth of root hairs by repeated readings at short intervals of time, is that of R. E. Jeffs (6) in our laboratory. He found, using a wide range of candle powers for various periods of time, that alterations in light have no effect upon the rate of elongation. With respect to temperature, however, he found that root hairs are very sensitive. A slight rise or fall in temperature causes complete cessation of growth for the time being, at least; and, if it is sufficiently marked, the stoppage is permanent. The method which Jeffs used for mounting his specimens was one employed by Blaauw (2) in his study of root-elongation. Seidel (19) used the same type of damp chamber more recently. A brief description of the mounts has been published (3).

While this method lends itself well to the study of the effects of light and temperature, it is not suited to the investigation of the effects of many conditions which may modify cell-enlargement, inasmuch as it involves growth in a gaseous medium. For the purpose of studying the effects of substances in solution, root hairs must be grown in a liquid. In their studies on root hairs in water, Zacharias (25) in 1891 and Sokolowa (21) in 1897 merely transferred the root from the culture to a slide and mounted it with water and a cover glass in the ordinary way. Stiehr (22) in 1903 used hanging-drop cultures. Osterhout (9) in 1910 devised a method of attaching a cover glass to a slide. A seed was then pushed under the cover glass and held tight by the latter while it germinated and developed root hairs. This method has the distinct advantage of making it possible readily to transfer the root hairs from one solution to another, and also to the microscope for observation. It, however, does not permit of continuing the experiment for a long period of time, and the effects of exposure on the microscope stage make it open to some objections for such studies as involve the taking of readings at frequent intervals. Osterhout's method was employed also by Seidel (19) in his study of the tropisms of root hairs.

For the present study water chambers were employed, which resemble in many respects the damp chambers used by Blaauw, Jeffs, and Seidel, referred to above. However, instead of the two glass slides being separated by a filter-paper frame, their margins were fastened together with sealing wax or de Kotinsky's cement in such a way that a small space was left between. By means of this water chamber the root hairs could be continuously observed for several days, if necessary, with the compound microscope, using either a 16-mm. or an 8-mm. objective.

The study of the effects of solutions on the rate of root-hair elongation necessitates the choice of a plant which produces root hairs luxuriantly in water. Schwarz (18) listed 27 species of phanerogams which do not produce root hairs in either water, air, or soil. He names 22 which have root hairs in air, but none, or only an occasional one, in water. He gives the names of only 10 which produce root hairs in water. Zacharias (25) in 1891 studied the root hairs of *Chara* and *Lepidium sativum* in water. Sokolowa (21) in 1897 used the seedlings of four species and the cuttings of two, *Azolla caroliniana* and *Tradescantia albiflora*, in water. Stiehr (22) in 1903 employed 5 species in water. Osterhout in 1910 (9) reported experiments in which 6 different genera were used in water, and in 1913 (10) he added three more. Schaede (17) in 1923 reported on the effects of dyes on the root hairs of *Hydrocharis morsus-ranae* grown in solution. This species was grown in water by Küster (7), who also used three other aquatic species for a study of root hairs in water. The first work on root-hair production in water was that of Perseke (11) in 1877. He found two species which produce root hairs as well in water as in soil, seven which produce fewer or shorter root hairs in solution than in damp air, and seven which

produce only rudimentary or isolated root hairs in water. Miss Roberts (15) studied radish root hairs in water. Miss Snow (20) used three species: corn, sunflower, and Windsor bean. Bardell (1) mentions three. Hill (5) used three halophytes. The most recent work is that of Seidel (19), based on the tropistic responses in aqueous media of root hairs of 19 species belonging to 11 families of angiosperms. Altogether at least 56 species have thus far been named in the literature as producing a considerable supply of fair-sized root hairs under water.

In 1905 Osterhout (8) recommended the use of *Tradescantia fluminensis* for the study of root hairs in water. In 1913 Rigg (14) used this plant in a study of the effects of bog water as compared with water from open lakes and springs upon the production of root hairs. He found that in bog water the root hairs are stunted, in fresh water they are normal, and in water from drained or partly drained bogs they are also normal. He also exposed the root hairs to dilute solutions of sea water, formalin, tannic acid, gelatin, coffee, and tea, and obtained stunting effects in these media also. Concentrating the bog water by boiling increases the effect. While not primarily interested in the effects of different solutions on root-hair production, Osterhout (9) reported in 1910 that they "grew nearly as well" in distilled water as in tap water or Knop's solution.

The species used in the present investigation was *Tradescantia fluminensis*. The readings for the respective root hairs observed are given below as series A, B, C, etc. The tap water used came originally from a well 800 feet in depth, and was drawn from the University mains. The Knop's solution employed was made according to the following formula: 0.25 g.  $\text{KH}_2\text{PO}_4$ , 1 g.  $\text{Ca}(\text{NO}_3)_2$ , 0.25 g.  $\text{MgSO}_4$ , 0.12 g.  $\text{KCl}$ , a trace of  $\text{FeCl}_3$ , 1000 cc. of water. Series A to N inclusive were grown in tap water, Series O to Y inclusive in Knop's solution. The experiments of series A, B, and C were carried out in the research room of the plant physiological laboratories. This is in the basement and has cement walls, ceiling, and floor, with two small windows facing a near-by building. A daylight micro-lamp was used for making the readings and turned off between them. In series B the ceiling lights of the room were left on continuously; in series A they were on except from 11 P.M. to 8:30 A.M. In series C they were not on at all. Series D to N inclusive were run in the dark room adjoining the research room. This is a similar room, without windows, and ventilated by a fan drawing air through the winding entrance. No light was permitted in the room during these observations, except a continuous weak red micro-lamp used for taking the readings. Series O to V inclusive were done in the constant-temperature room. This adjoins the dark room and is similar, except that it is unventilated, has no artificial heating system, and is entered by a vestibule involving two doors. The temperature here, as in other cases, was about 22° C. and was constant throughout any series. The illumination was the same as in the dark room. The roots studied in Knop's



solution were grown first in tap water, and were transferred to Knop's solution at least 24 hours before the observations were begun. Thus the entire life of the root hair studied was spent in Knop's solution. Series *R*, *S*, *T*, and *U* were of different root hairs on the same root; as were also series *X* and *Y*.

Series *A* began at 3:30 P.M. and ran until 6:10 P.M. of the following day. Readings were taken at ten-minute intervals until 12:30 A.M. Thereafter readings were taken at 8:30, 8:40, and 10:00 A.M., at noon, and at 1:30 and 6:10 P.M., and the elongation of the root hair was calculated per ten-minute interval. All readings given below in all series are reduced to the scale of the elongation in microns per ten-minute interval. The readings for series *A* are: 12, 12, 12, 12, 16, 16, 16, 16, 16, 12, 20, 12, 16, 16, 16, 16, 16, 12, 16, 12, 16, 12, 12, 8, 16, 12, 12, 12, 12, 8, 12, 12, 12, 8, 12, 12, 12, 12, 8, 12, 8, 8, 8, 12, 12, 8, 12, 8, 8, 8, 8, 8, 8, 5, 3.3, 4, 4. The italicized figures indicate the increase in length per ten-minute period prorated from readings taken more than the usual interval apart.

Series *B* ran from 4 P.M. until 10:40 P.M., with readings at ten-minute intervals as follows: 20, 8, 12, 12, 8, 12, 12, 12, 12, 12, 12, 12, 8, 12, 16, 8, 12, 12, 12, 8, 12, 16, 16, 8, 8, 8, 0, 0, 0, 0, 8, 4, 4, 4, 0, 8, 4, 4, 0. No reading was taken in this series at 9:40 P.M.

Series *C* extended from 10:40 P.M. to 12:30 P.M., with subsequent readings at 8:30, 8:40, and 10:10 A.M., noon, and 1:30 P.M., as follows: 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 8, 11, 5.8, 12.6.

Series *D* extended from 8 A.M. to 12:20 P.M. with all except the first two readings at 20-minute intervals; as before, however, the record shows the prorated growth per ten-minute interval: 4, 4, 4, 6, 8, 8, 4, 6, 6, 8, 6, 8, 2, 2.

Series *E* is taken in a like manner from 2 P.M. until 10:20 P.M.: 4, 4, 12, 8, 8, 8, 8, 8, 16, 8, 6, 6, 6, 6, 10, 10, 10, 10, 10, 10, 10, 10, 10, 10, 9. The final reading, taken at 7:20 A.M., shows that over night the root hair continued to grow at an average rate of about 9 microns per ten-minute interval.

Readings in series *F* were taken at 7:30, 7:40, 8, 9, 10, and 11 A.M., noon, 1:30, 2:50, 4:10, and 5 P.M. as follows: 5.1, 5.1, 4.7, 3.3, 5.3, 4, 3.7, 4, 5, 6.3.

Readings in series *G* were taken at the same time as in series *F*: 5.1, 5.1, 5, 9, 10.8, 10.8, 8, 11, 9, 8.

Readings in series *H* were taken continuously from 2:40 to 5:10, and thereafter at 5:30, 6:10, 6:20, 6:40, 7, and 9 P.M., and again at 8 A.M., as follows: 16, 16, 4, 20, 12, 8, 12, 12, 16, 16, 12, 16, 16, 16, 16, 14, 11, 12, 12, 12, 12, 8.

Readings in series *I* were taken every ten minutes from 2:40 until 5:10, and thereafter every 20 minutes until 10:10 P.M., except that no reading was taken at 5:50. The final reading was then taken at 8 A.M.: 16, 16, 4,

20, 12, 8, 12, 12, 16, 16, 12, 16, 16, 16, 8, 14, 13, 12, 10, 14, 12, 12, 12, 12, 12, 4, 20, 12, 6, 7.4.

Series *J* was taken every fifteen minutes from 10:30 A.M. until 12:30 P.M.: 5.4, 10.8, 5, 4, 10.8, 0, 0, 0, 0. In this and subsequent series in which the readings were taken every fifteen minutes and the record is given per ten-minute interval, italics will not be used, except for longer intervals than fifteen minutes.

Readings in series *K* were taken every fifteen minutes from 7:15 A.M. until 1:30 P.M., except that no reading was taken at 10 A.M.: 10.8, 12, 10.8, 16.2, 5.4, 16.2, 5.4, 16.2, 10.8, 10.8, 5.4, 10.8, 5.4, 10.8, 5.4, 8.1, 8.1, 5.4, 5.4, 5.4, 5.4, 10.8, 5.4, 5.4.

Series *L* was taken every 15 minutes from 3 to 4 P.M.: 16.2, 5.4, 10.8, 5.4.

Series *M* was from 5:00 to 5:30: 5.4, 16.2.

Series *N* lasted from 1:45 to 5:30 P.M. with readings taken every fifteen minutes: 16.2, 10.8, 10.8, 16.2, 10.8, 21.6, 10.8, 21.6, 16.2, 16.2, 16.2, 16.2, 16.2, except that no readings were taken at 4:15 or 4:30.

Series *O* to *V* inclusive in Knop's solution were taken with readings for the most part (except where noted in italics) at fifteen-minute intervals. Readings in series *W*, *X*, and *Y* were taken every ten minutes.

Series *O* runs from 2 to 4 P.M.: 5.4, 10.8, 0, 5.4, 5.4, 5.4, 5.4, 5.4.

Series *P* runs from 9:15 to 11:45 A.M., with no reading at 11 A.M., and with a final reading at 1:45 P.M.: 10.8, 5.4, 5.4, 0, 5.4, 5.4, 5.4, 2.7, 5.4, 5.4, 5.4.

Readings in series *Q* were taken at the same time as in series *P*: 5.4, 5.4, 0, 5.4, 10.8, 0, 5.4, 2.7, 5.4, 5.4, 4.05.

In series *R*, *S*, *T*, and *U* readings were taken every fifteen minutes from 1:45 to 4 P.M., and thereafter at 5 and 6 P.M.:

*R*: 10.8, 0, 5.4, 5.4, 5.4, 5.4, 2.7, 5.4, 2.7, 4.05, 5.4.

*S*: 8, 5.4, 5.4, 5.4, 5.4, 5.4, 5.4, 2.7, 2.7, 5.9, 4.05.

*T*: 5.4, 5.4, 2.7, 8, 5.4, 0, 5.4, 5.4, 5.4, 5.4, 4.05.

*U*: 5.4, 5.4, 5.4, 5.4, 5.4, 5.4, 2.7, 5.4, 2.7, 5.4, 2.7.

Readings in series *V* were taken every fifteen minutes from 9 until 11:15 A.M. with an omission at 10:30, and final readings at noon and at 7 A.M.: 5.4, 10.8, 5.4, 2.7, 8, 2.7, 5.4, 5.4, 3.6, 5.4.

Readings in series *W* extend from 8:40 until 11:40 A.M.: 3.5, 3.5, 3.5, 3.5, 3.5, 3.5, 3.5, 3.5, 3.5, 3.5, 3.5, 0, 3.5, 0, 7, 0.

Readings in series *X* were taken at the same time as those of series *W*: 4.7, 7, 0, 7, 0, 7, 3.5, 3.5, 3.5, 3.5, 3.5, 0, 3.5, 3.5, 0, 0, 3.5, 3.5.

Readings in series *Y* extend from 8:40 until 11 A.M.: 3.5, 3.5, 0, 7, 0, 3.5, 3.5, 0, 3.5, 3.5, 3.5, 0, 3.5, 3.5.

The data obtained in these experiments show a marked difference in the rate of growth of root hairs of *Tradescantia fluminensis* in tap water, as compared with those in Knop's solution. The average growth per

TABLE I. Summary of Data in Series A to N

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	Aver.
Initial length in microns.....	48	32	56	64	48	184	56	152	206	120	36	64	240	56	
Date of observations.....	1/28	1/28	1/28	1/30	1/30	1/31	1/31	2/1	2/1	3/15	3/15	3/16	3/16	3/16	
Time of setting up culture.....	9 A.M.	9 A.M.	9 A.M.	1/28	1 P.M.							1:45 P.M.	1:45 P.M.	1:45 P.M.	
Total period of observation (min.).....	1:332	528	890	260	1:040	575	575	960	1,040	60	368	60	30	225	
Total elongation (microns).....	1,300	336	832	152	896	248	512	968	992	48	324	56	32	344	
Average elongation in microns per min.....	1	0.636	0.935	0.584	0.861	0.431	0.88	1	0.95	0.8	0.88	0.93	1.06	1.53	0.916
Elongation during															
first hour.....	80	72	48	28	48	29.4	30.6	88	76	48	72			80	
second hour.....	92	72	48	40	48	24	42	56	84	0	64			96	
third hour.....	92	64		40	64	25.4	59.4	64	79		56			96	
fourth hour.....	76	60		32	40	27.9	64.8	63	50		48				
fifth hour.....	72	16			44	23.1	56.4	71	74		40				
sixth hour.....	68	24			60	22.2	56		62		40				
seventh hour.....	60				60	24	66		72		40				
eighth hour.....	52				60	28	58								
ninth hour.....						32.6	52								
third to eleventh hour.....			60												
tenth hour.....															
During last hour.....							48								
During night.....	48	20	75	24	54	36.5		48	44.4	48				96	
In forenoon, second day.....	30														
In afternoon, second day.....	24														
Average elongation in microns per hour.....	59	38.16	56.1	35	51.66	25.81	52.8	60	57	48	52.8	54		91.8	

minute in tap water was 0.916 micron. The average growth per minute in Knop's solution was 0.437 micron. The fastest growth recorded in tap water during any ten-minute period was 24 microns; while in Knop's solution it was 10.8 microns. The maximum average growth per hour for any series in tap water was 91.8 microns; while in Knop's solution it was 32.98 microns. It is thus evident that growth of root hairs in tap water is from twice to three times as fast as in Knop's solution.

TABLE 2. *Summary of Data, Series O to Y*

	O	P	Q	R	S	T	U	V	W	X	Y
Initial length....	56	48	72	48	60	88	56	96	28	117	21
Date.....	3/17	3/17	3/17	3/17	3/17	3/17	3/17	3/25	3/29	3/29	3/29
Total period....	120	290	290	255	255	255	255	1320	180	180	140
Total elongation..	64	144	120	128	124	120	112	704	56	57.2	38.5
Average elongation per min. ...	0.533	0.497	0.414	0.5	0.485	0.47	0.47	0.533	0.311	0.318	0.275
Elongation											
first hour.....	32	32	24	32	36	32	32	36	21	25.7	17.5
second hour...		28	28	28	28	24	28	30	21	17.5	14
third hour.....		28	26	28	36	32	32	24	14	14	
last hour.....	32	32	24	32	24	24	16				17.5
Average elongation per hour ..	32	29.82	24.84	30	29.1	28.2	28.2	32.98	18.66	19.08	16.5

Final average elongation per minute: 0.437 micron.

Whether this is to be regarded as the stimulating effect of some non-essential radicals in the tap water, or as the toxic effect of Knop's solution, is at present a matter only of speculation. It serves, however, to show that not only may substances in solution modify the form of root hairs, as Schwarz (18) found, or cause them to burst, as Prowazek (12) found for the root hairs of *Hydrocharis* in 1907, or alter their suction force, as Ursprung and Blum found in 1921, or their production, as Stiehr (22) found in 1903, or their direction of growth, as Seidel (19) has recently discovered, or their cyclosis, as found by Sokolowa (21) in 1897, but also their rate of elongation. This result thus forms a basis for further study upon the effects of distinct chemical compounds and ions upon the rate of cell-enlargement.

This series of readings made on 25 different root hairs of *Tradescantia fluminensis* reveals no such S-shaped grand period of growth as Reed (13), Robertson (16), and others have found for dry-weight increase, stem-elongation, increase in leaf size, etc., which they have presented as a basis for the interpretation of growth as an autocatalytic phenomenon. Jeffs (6) found an S-shaped grand period of growth in the life history of the root hairs of corn and white mustard. In these plants the rate of root-hair elongation is accelerated during the first hour or so, then remains constant for several hours, and then slowly is retarded to zero again over a period of an hour or more. Jeffs, however, interprets the acceleration as due to

cellular interaction, retarding the elongation of the epidermal cell vertically and resulting in its projection laterally in the form of a root hair, a constant increase in volume thus being maintained.

Series *A* of this study shows a slight acceleration at first covering a period of about an hour, followed by a period of slow retardation lasting for six hours at least and perhaps for 24 hours. Series *B* shows a steady retardation to zero with minor fluctuations over a period of six hours. Series *C* shows a constant rate of growth for two hours, and probably for ten hours, with an acceleration thereafter. Series *D* has somewhat of an S-shaped curve over a period of about four hours. Series *E* shows an initial acceleration during the first hour with a gradual retardation during four hours almost to the original rate, followed by a constant, relatively rapid rate for four hours. In series *F* and *G* the readings are at longer intervals but indicate a more or less constant rate throughout. Series *H* shows rapid fluctuations, with a rather constant hourly average however. Growth in series *I* was also approximately constant, but somewhat slower near the middle of the eight-hour period. Series *J*, *L*, *M*, and *O* are too short for consideration in this connection. Series *K* approaches somewhat the S-shaped curve, especially as to the retardation in the latter part. Series *N* gives the reverse situation, with the slower growth near the middle. Series *P*, *Q*, *R*, *S*, *T*, *U*, *V*, *W*, *X*, and *Y* show an approximately constant rate throughout. It thus appears that the rate of cell-enlargement of the root hairs of this plant tends to be uniform throughout their life history rather than to follow the S-shaped-curve.

Whether this difference between this study and that of Jeffs is to be attributed to the use of a liquid medium as opposed to that of the damp chamber, or is a specific difference in the plants used respectively, is not as yet certain. However, it is interesting to note that no lateral movement of root hairs of *Tradescantia fluminensis* occurs, that is, the region in which the root hairs of this plant appear is entirely above the region of elongation of the root as a whole. There is thus no cellular interaction which might be referred to as a cause for an initial acceleration. The absence of such an acceleration thus serves to support further the interpretation of Jeffs, that lateral movement and acceleration are correlated and are different phases of the same phenomenon. The root hairs of this plant elongate at a nearly constant rate, thus accomplishing increase in volume of the epidermal cells at a constant rate; while in corn and in white mustard increase in volume is kept constant by the acceleration of root-hair elongation to compensate for retardation in the vertical direction.

Some of the fluctuations in growth rate indicated by the readings given in the tables are doubtless due to errors in reading, rather than to actual variation in rate of elongation of the root hair from time to time. With the magnification employed there was a possible error of about 2 microns in each reading. Should such an error be made in one reading it would

perhaps be corrected in the next, thus making a variation of 4 microns. A number of such examples may be found in the tables, where there is an apparent drop followed by a rise, or *vice versa*, and then a return to normal.

The wider fluctuations, however, together with the apparent entire cessation of growth during some ten-minute periods, as appears in series *W*, *X*, and *Y* for instance, indicate that the root hair may actually grow by pulsations, rather than at a steady rate. Jeffs (6) found evidence of this in the root hairs which he studied. He found that the hairs do not have perfectly cylindrical shapes, but that they curve backward and forward in a rather zigzag fashion, which is not apparent without careful observation. This shape he thought perhaps due to a periodic cessation of growth and a shifting thereafter of the apical point to one side or the other. Evidence of this was found when elongation was caused to cease by changes in temperature. When growth was resumed thereafter it sometimes took a direction practically at right angles to the preceding direction of growth, resulting in an L-shaped root hair.

Observations on the root hairs of *Tradescantia fluminensis* strongly support this view. Root hairs were observed which normally turned their apical point and grew for a time almost at right angles to their preceding direction of growth. This curvature was commonly accompanied by an apparent retardation of growth as shown by the readings taken, as, for instance, the retardation at 9:40 A.M. in series *D*, at 4:45 P.M., 7:15 P.M., and 7:45 P.M. in series *B*, and at 7:55 A.M. and 11:55 A.M. in series *F*. After growing a short distance in another direction, that is, at about right angles to the normal direction, another change in direction occurs, bringing the root hair back to its normal orientation. These changes leave kinks in the root hair which persist for a time very prominently, but become more and more smoothed out until they are almost obliterated. Schwarz (18) noted modifications in the form of root hairs effected by nutrient solutions, but those seen here are not at all of the type he describes and figures. These do not involve a change in the diameter of the root hair at any point, but only one of direction of growth. Neither do they resemble the tropistic movements described by Seidel (19) as resulting from chemical stimulation. They most nearly approach the changes in direction described and figured by Stiehr (22) as resulting from electrical excitation.

Haberlandt early (4) noted the tendency of the nucleus of the root hair to lie near the tip. He regarded this as indicative of the close relationship of the nucleus to growth processes, especially cellulose-deposition, inasmuch as he also then for the first time determined that the root hair elongates by apical growth. A quite recent work by Windel (24) done in his laboratory gives further evidence of the relationship of the position of the nucleus to this function. He studied *Sinapis alba* and found that the nucleus moves from the base to the apex of the root hair where growth is most active. Sokolowa (21), however, does not believe that the nuclear position is related

TABLE 3. *Position of Nucleus in Root Hair*

Readings in tables 3 and 4 are in micrometer spaces, which are each eight microns.  
First column in each instance, length of root hair; second column, distance of nucleus from base of hair.

A		B		C		D		E	
39	32	19	10	11	5	11	4	26	17
41	35	31.5	21.5	13	6	12.5	6.5	32.5	23
42.5	35	32.5	22	14	8	14.5	7.5	34	25
44.5	38	34	25	15	8.5	16.5	11	35.5	27
46	41.5	41	34	16	9	17.5	11	38	29
47.5	43			17	9	19	11	43	35
48.5	42			18	9	20.5	14	63	54
50.5	44			75	65	22.5	14		
52	44					24	18.5		
55	47					26	20		
56.5	49					26.5	18		
57.5	51.5								
59	51.5								
60.5	55								
63	57								
64.5	63								
66	63								
67.5	63								
69	63								
73.5	61								
79	74								
81.5	76								
83	77								
84.5	79								
85.5	81								
86.5	81								
87.5	84.5								
139	130								
140	133								

F		G		H		I	
23	18	9	3	19	11	26	21
25	19	21	11	21	16	28	21
28.5	22	51	36	22	18	30	20
41	38	66	56	25	19	30.5	24
		82	60	26	16	33	31
				28	17	34.5	26
				30	20	35.5	29
				30.5	22	37	30
				32	24	38.5	30
				33	25	40.5	30
				34.5	27	42.5	34
				35.5	26	44	35
				37	26	46	36
				38	26	48	37
				40	30	50	37
				41	31	51	40
				43	31	86	78
				45	32	91	81.5
				47	36	94	85
				80	70	95.5	87
				140	130	150	145

to surface enlargement of the cell wall. He found that plasmolyzed hairs deposit cellulose some distance from the nucleus as well as near it.

In the present study some observations were made on the position of the nucleus with respect to the tip of the root hair. The nucleus moves into the root hair soon after the emergence of the latter, and remains a short distance behind the tip throughout the growth period of the hair. Table 3 gives the location of the nucleus in specimens *A* to *I* inclusive, the growth rates of which are given in series *A* to *I*. In the first column in each instance is given the length of the root hair at the time of the respective successive readings. In the second column is given the distance of the nucleus from the base of the root hair at the time the reading was taken. The difference between the two readings thus gives the distance of the nucleus from the tip of the hair.

TABLE 4. *Position of Nucleus in Root Hair*

Measurements of a number of root hairs, not used for serial studies.

Length of root hair.....	82	140	129	85	116	78	175	50	57	73
Distance of nucleus from base.....	60	132	125	67	114	70	170	25	36	67

From tables 3 and 4 it will be seen that the nearest that a nucleus was seen to approach the tip of a root hair was 12 microns; and the greatest distance that a nucleus was found from the tip was 200 microns. The average distance was 63 microns. In some root hairs the distance of the nucleus from the tip seems to be nearly constant, as in *E*. In others the distance varies from time to time. At times the nucleus seems to move forward faster than the tip of the root hair, at other times it moves at the same rate or more slowly. Some readings indicate that it may even move a short distance in the direction of the base of the hair. No relationship can be found from these data between the position of the nucleus and the rate of elongation of the root hair. The nucleus in a root hair which is growing rapidly is in general no nearer to nor farther from the tip than the nucleus in a root hair that is growing more slowly.

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# THE EFFECT ON PLANTS OF THE INCREASE AND DECREASE OF THE PERIOD OF ILLUMINATION OVER THAT OF THE NORMAL DAY PERIOD

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## INTRODUCTION

Garner and Allard (3) have shown that the relative length of day and night may be an important factor in the development of plants. They found that certain plants attain the reproductive stage only when exposed to a relatively short day and become more strongly vegetative with increased length of day; while other plants require a relatively long day to reach the reproductive stage and become weakly vegetative with a shorter day. The problem at hand was to repeat and if possible to confirm a portion of the work of these authors and to make anatomical and physiological studies in an attempt to throw light on the problem of how the length of day influences the form of plant-development.

## EXPERIMENTS

The species used in these experiments consisted of the tomato (*Lycopersicum esculentum* Mill.) and the pepper (*Capsicum annuum* L.), the former being chosen in order to compare with Kraus and Kraybill's (8) experiments on tomatoes. Pepper seeds of the Large Bell or Bull Nose variety were planted on September 16, 1921, and tomatoes of the Bonny Best variety were planted on October 14, in flats containing sandy loam. On November 22 they were transplanted to boxes containing loam enriched with a goodly quantity of sheep manure. There were three boxes of each kind of plant, and beginning on November 30 they were subjected to different lengths of day. One set was given a short day of  $6\frac{1}{2}$  hours by placing them in a ventilated dark chamber at 4 P.M. each day and removing them at 9:30 A.M. One set was given a long day by supplementing the daylight by an electric light, using a "daylight" 100-watt nitrogen bulb suspended above the plants and turned on from 4 to 11 P.M. and from 5:30 to 9:30 A.M. The third set received the normal day period. All plants were grown in the greenhouse under similar conditions of temperature, humidity, etc.

Records were kept of the growth in height, date of blossoming, number of flowers produced, and the number of fruits set.

The growth in height in both the tomato and the pepper plants varied to a

considerable extent under the different lengths of day, and, although usually plant stems elongate to a greater extent when in darkness, such was not the case in this experiment. On March 6 the average heights of the pepper plants were 6 inches in those exposed to the short day, 16 inches in the control plants (exposed to normal day period), and 29 inches in those with the long day. The tomato plants exposed to the short day averaged 41 inches in height, the controls averaged 56 inches, and those exposed to the long day averaged 68 inches. The rate of growth is thus shown to vary directly with the length of day, which is in accordance with the results of Garner and Allard (3) and those of Adams (1).

The tomatoes with the long day blossomed sooner than the other two sets and also produced more flowers. This shows that tomatoes are long-day plants, meaning (according to Garner and Allard) that for the best seed-production a daily period of illumination of 12 hours or more is required. Those with the short day did produce a few flowers, but did not set any fruit. During November and December, when the days were short and often cloudy, the controls were more nearly like the short-day plants, but as the days lengthened during January and February their rate of growth increased so that by March 7th they bore a greater resemblance to the long-day plants.

The peppers with the short day made very little growth and no flowers were produced. They were very pale in color in comparison to the other two sets. The peppers with the long day blossomed at practically the same time as the controls, although they were more than twice as high as the latter at that time. Both sets had produced buds previously but they had dropped off before opening, probably because of the high percentage of nitrogen in the soil. But the controls produced more blossoms than the long-day plants, and also set more fruit, seeming to indicate that too long a day is unfavorable for seed-production in peppers.

As a part of this investigation a preliminary experiment was carried on during the summer of 1921, which consisted in a series of planting of nasturtium (*Tropaeolum minus* L.) made every five days out of doors from June until September. Those planted early in June produced their first flower after an average vegetative growth-period of 59 days. This period was gradually shortened during the summer until those planted July 27 blossomed after 41 days. In the plantings made after that date the growing period increased in length, until those planted August 16 required 54 days to attain the flowering stage.

As the length of day decreased the nasturtiums had a shorter growing period, but undoubtedly temperature was also a factor. The lower temperatures prevailing in the fall would retard growth, and this evidently explains the slight lengthening of the growing period in the autumn. On the other hand, during the summer, while the days were becoming shorter, the warmer temperatures may have stimulated more rapid development and in this

way might have aided in shortening the growing period. The general conclusion to be drawn from this experiment is that the nasturtium is what Garner and Allard speak of as a "short-day" plant; that is, it makes its best development when the daily exposure to light is of less than 12 hours' duration.

#### ANATOMICAL STUDIES

The leaves of the plants grown under the different lengths of day varied a great deal in thickness, and the average thickness in each series was determined by means of measurements made with an eye-piece micrometer. The leaves of the tomatoes averaged 0.150 mm. in the short-day series, 0.167 mm. in the controls, and 0.200 mm. in the long-day series. The peppers averaged 0.133 mm. with the short day, 0.153 mm. with the normal day period, and 0.167 mm. with the long day. As will be seen from these data, the shortening of the daily period of illumination was correlated with a decrease in the thickness of the leaf, and with the long day there was always an increase in thickness. The relative number of chloroplasts as shown in cross sections of the leaves was observed to be directly proportional to the amount of light, and the variation was so great that it was quite apparent in the color of the leaves.

In the anatomical studies of the stems, comparisons were always made of sections of the same age. In the stems of the tomatoes the maximum diameters were 9 cm. in the short-day series, 11 cm. in the controls, and 13 cm. in the long-day series. The stems of the peppers with the short day had a maximum diameter of 3.5 cm., while the controls and the long-day series each had a maximum diameter of 7 cm.

The epidermal cells were found to vary in size in the different series. They were smallest in the short-day and largest in the long-day series. In the latter, cork was beginning to form in the oldest stems, but none was present in the other two series, as shown in Plate XXXIX. The short-day series had comparatively more pith and less xylem and bast, and the walls of the xylem and bast cells were much thinner than in the long-day series. For example, in the oldest stems of the short-day series, a stem 9 mm. in diameter had a maximum thickness of xylem of 1.2 mm. In the long-day series a stem 11 mm. in diameter had a maximum of 2.1 mm. of xylem, an increase of about 6 percent over the short-day series, relatively speaking. There were fewer large vessels, but the average opening was a little larger in the short-day series, as is evident in an examination of the photomicrographs of Plate XXXIX. The controls were always intermediate in all the above-mentioned respects between the other two sets.

The results with the peppers were very similar to those obtained with the tomatoes. The plants with the long day had a relatively large amount of cork-development, while the other sets had only a small amount. The long-day series also had comparatively more xylem, but less cortex and pith. The amount of bast did not vary much, but the walls were thicker with the

long day, and the walls of the xylem cells were thicker in about the same proportion.

#### PHYSIOLOGICAL EXPERIMENTS

Since the plants were grown under such varied conditions of light, it seemed likely that an expression of this factor might be found in the density of the cell sap. The concentration of the cell sap is proportional to its osmotic pressure, and this may be determined conveniently by making freezing-point determinations. The materials used consisted of the leaves of the control plants and of the long-day plants, and in the case of the tomato the fruits also were used, which at this time averaged about one and one half inches in diameter and were still green. No freezing-point determinations could be made of the plants with the short day, because in the case of the peppers there was not sufficient material and the tomatoes were accidentally destroyed.

The parts to be tested were first ground up in a meat-chopper and placed in a large test tube, which was then placed in a Dewar flask containing solid  $\text{CO}_2$  mixed with ether, the purpose of the freezing being to render the membranes permeable so that the juice obtained by mashing this pulp would approach more nearly in concentration the actual concentration of the sap. The freezing points were determined by means of the Bartley freezing-point apparatus, using a Beckman thermometer. From five to ten readings were made of each sap, readings being made to one thousandth of a degree. The results are shown in table I.

TABLE I

	Tomatoes				Peppers	
	Leaves		Fruit		Leaves	
	Control	Long Day	Control	Long Day	Control	Long Day
Average depression of freezing point ( $\Delta$ ).....	0.830° C.	0.841° C.	0.975° C.	0.919° C.	0.931° C.	0.995° C.
Osmotic pressure at freezing point.....	7613.76 mm.	7705.49 mm.	8943.87 mm.	8430.17 mm.	8540.25 mm.	9127.33 mm.

$P_f = 9173.2\Delta f$  was the formula used in the determination of the osmotic pressure at the freezing point, in which  $P_f$  is the osmotic pressure at the freezing point of the solution in millimeters of mercury, and  $\Delta f$  is the difference between the two freezing points of the material examined compared with that of pure water. The results obtained indicate that the plants with the long day contained more concentrated sap than the control plants. In the case of the fruits of the tomato, diametrically opposite results are indicated; in other words, the cell sap of the fruits on the controls was more concentrated than that of the fruits of the long-day plants.

Since the osmotic pressure of the cell sap of plants may be affected by a number of factors, an interpretation of the above-quoted results can hardly be attempted until further data are obtained. The concentration of the cell sap, however, was proportional to the length of day and to the amount of starch. It may be, therefore, that the greater concentration of the sap in the long-day series is due to a greater concentration of soluble carbohydrates.

#### MICROCHEMICAL TESTS

Starch was the only substance tested for microchemically in these experiments. The ordinary iodine test was employed, using sections of stems which had been preserved in formalin alcohol (the material having been harvested during the afternoon, between 2 and 3 o'clock). The amount present was found in every instance to be directly proportional to the length of day.

#### CHEMICAL ANALYSES

Kraus and Kraybill (8) in their work on the nitrogen-carbohydrate ratio were able to show by means of chemical analyses a definite relation between the form of development in the tomato and the proportion of nitrogen to carbohydrates. Riede (11) also has shown a correlation between the development of the plant and the quotient represented by the ratio of carbon assimilation to absorption of inorganic salts. He found that in *Zea Mays* the quotient must be higher for the production of pistillate than for that of staminate flowers. Harvey and Murneek (6, 7) found that the behavior of apple spurs is influenced by the carbohydrate-nitrogen ratio. Nightingale's (9) results in chemical studies of plants grown under different lengths of day also seem to indicate a definite carbohydrate-nitrogen relationship between the type of growth and the chemical composition. Therefore it would seem that interesting data might be obtained by comparing the nitrogen-carbohydrate ratios in the experiments under discussion. Chemical analyses have not as yet been possible, but samples of the material have been preserved for making such determinations.

#### GENERAL DISCUSSION

The relation between the time of flowering and the comparative length of day and night was brought out clearly for the first time in Garner and Allard's (3) recent work, and this theory offers explanations of a number of previous observations which at the time could not be interpreted. Palladin (10), in his discussion of the dependence of growth and configuration upon temperature, gives the dates of flowering of certain plants at Brussels and at Petrograd and makes an attempt at explanation on the basis of the summation of the daily temperatures. He found that at Petrograd the plants came to flower with a much smaller temperature summation than at Brussels and, not being able to give any explanation of this, he merely concludes that

"the life of the plant is thus not governed entirely by the amount of heat received; the internal conditions of the plant must also be considered." It is now apparent that the difference in the length of day of these different locations is an additional important factor.

The results obtained in my own experiments are in agreement with those of Garner and Allard (3). As they reported, the rate of growth in every case was proportional to the length of the daily exposure to light, but in the development of flowers the various types of plants had different light requirements. The tomato made its best growth when subjected to a very long day. The peppers made the best development in the normal day period, which at that time of year was comparatively short. The preliminary experiment with nasturtiums showed that they were able to shorten their growing period a little as the days shortened.

Either vegetative development or production of flowers could be produced by regulating the daily exposure to light, and similar results were obtained by Kraus and Kraybill (8) by varying the amounts of available nitrogen. While the writer was carrying on this research along the lines of Garner and Allard's work, Miss Eltinge (2) was working along the lines of Kraus and Kraybill's problem, using the same species of plants, tomato and pepper, in order that comparisons could be made.

The writer found that the amount of starch was directly proportional to the amount of light, and Miss Eltinge found that the amount varied inversely with the amount of nitrogen. In general appearance the plants which were subjected to the short day (9:30 A.M. to 4:00 P.M.) resembled those grown without nitrogen, all being very small and undeveloped and pale and yellowish in color. In the microchemical analysis for starch, however, the plants without nitrogen were found to have an extremely large amount, while those with plenty of nitrates but with the short daily exposure to light contained practically no starch. Yet the plants in the two cases resembled each other very closely in their slowness of growth and lack of vigor. These results go toward proving the first and last of the four general conditions given by Kraus and Kraybill concerning the relation between the nitrogen-carbohydrate ratio and the amount and type of development, which are:

(1) Though there be present an abundance of moisture and mineral nutrients, including nitrates, yet without an available carbohydrate supply vegetation is weakened and the plants are non-fruitful. . . . (4) A further reduction of nitrates without inhibiting a possible increase of carbohydrates makes for a suppression both of vegetation and fruitfulness.

Miss Eltinge found the greatest depression of the freezing point in the sap of the plants without nitrogen, while the writer found the greatest depression in the plants with the long day. It was observed that the osmotic pressure in every instance was proportional to the amount of starch present, and this fact points toward the conclusion that the increase in the sap-concentration in these cases was due to soluble carbohydrates.

This comparison further brings out the fact that the bast-development was not proportional to the amount or rate of growth but to the amount of starch. In the one case there were more bast cells and their walls were thicker in the stems of the plants subjected to the long day, which also were the plants to make the greatest amount of growth. In the other case the bast had made comparatively more development in those plants grown without nitrogen, consisting of those plants which made the slowest and the least amount of growth. The only observable factor to vary in the same way was the starch content. In other words, the bast-development seemed to vary directly with carbohydrate supply and bore no relation to the amount or rate of growth.

In both cases the amount of xylem varied directly with the amount of vegetative growth, the greatest amount always being present in the stems growing the most vigorously—those with the greatest amount of nitrogen in Miss Eltinge's experiments, and those exposed to the longest day in the writer's experiments. These results are opposed to those of Kraus and Kraybill, who found that the xylem was greatly reduced in vigorously vegetative stems.

The general conclusion to be drawn is that probably the different lengths of day influence the form of plant-development by changing the nitrogen-carbohydrate ratio.

#### SUMMARY

1. A series of plantings of dwarf nasturtiums was made out of doors at 5-day intervals during the summer, and as the days became shorter the plants attained the flowering stage sooner, so that the growing period of those planted in the first part of August was about 10 days shorter than the growing period of those planted in the middle of June.

2. During the winter, tomatoes and peppers were raised in the greenhouse and subjected to different lengths of day. One set had a long day (from 5:30 A.M. to 11 P.M.), a second received the normal day period, and the third series was given a very short day (9:30 A.M. to 4:00 P.M.). The long day was obtained by means of supplementing the daylight by an electric light, and the short day by means of a ventilated dark chamber.

3. The amount and rate of growth was directly proportional to the length of day. This was true of the height and diameter of the stems and of the size and thickness of the leaves.

4. The tomatoes which had the long day blossomed sooner than the other two sets and also produced more flowers and set more fruit. Peppers subjected to normal daylight produced more flowers and set more fruit than the other sets. With both the tomatoes and the peppers no fruit was obtained under the short day, and the peppers did not even produce flowers. Tomatoes might therefore be designated as "long-day" plants, while peppers require a moderately short day for the best seed-production.



5. Anatomical studies of the stems of the tomatoes and peppers raised under the various light conditions showed that the amounts of both bast and xylem varied directly with the length of day, and that the thickness of the walls of these cells varied in the same way. The size of the epidermal cells and the amount of cork-development also varied directly with the amount of light. The stems which were exposed to the short day contained relatively more pith.

6. The leaves were thickest and largest and contained the most chloroplasts on the plants exposed to the long day, and they were smallest, thinnest, and most pale in color in those exposed to the short day.

7. Freezing-point determinations of the cell sap of the leaves indicated the greatest concentration to be in the plants exposed to the long day, but in the case of the fruits of the tomato the sap was more concentrated in those exposed to a shorter day. In this experiment and also in Miss Eltinge's experiments on the carbohydrate-nitrogen ratio in these plants, the osmotic pressure in the leaves was always proportional to the amount of starch present rather than to the rate of growth, suggesting the possibility that the increase in sap-concentration in these cases might be due to soluble carbohydrates.

8. The amount of starch present in the stems was directly proportional to the length of day.

9. The bast-development in these stems was directly proportional to the amount of light; but, considering both these and Miss Eltinge's experiments, the amount of bast-development seemed to be proportional to the amount of starch rather than to be correlated with the rate of growth.

10. Probably the differences in the relative length of day and night influence the form of plant-development by a change in the nitrogen-carbohydrate ratio.

Most grateful acknowledgment is due to Dr. H. F. A. Meier of Syracuse University, who directed this research and gave generously of assistance and advice throughout the investigation; and to Dr. H. W. Browning of Rhode Island State College for valuable suggestions in the preparation of this paper.

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#### EXPLANATION OF PLATES

##### PLATE XXXVIII

Peppers (*Capsicum annuum* L.) grown under different lengths of day, as they appeared at the end of the experiment.

FIG. 1. Exposed to light from 9:30 A.M. to 4:00 P.M.

FIG. 2. Exposed to the normal day period.

FIG. 3. Exposed to light from 5:30 A.M. to 11:00 P.M.

##### PLATE XXXIX

Photomicrographs of cross sections of the oldest stages of stems.

Series A. Tomatoes (*Lycopersicum esculentum* Mill.).

Series B. Peppers (*Capsicum annuum* L.).

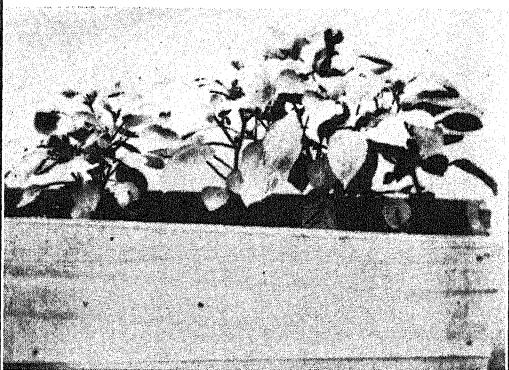
FIGS. A1, B1. From plants exposed to light from 9:30 A.M. to 4:00 P.M.

FIGS. A2, B2. From plants exposed to normal day period.

FIGS. A3, B3. From plants exposed to light from 5:30 A.M. to 11:00 P.M.



1.



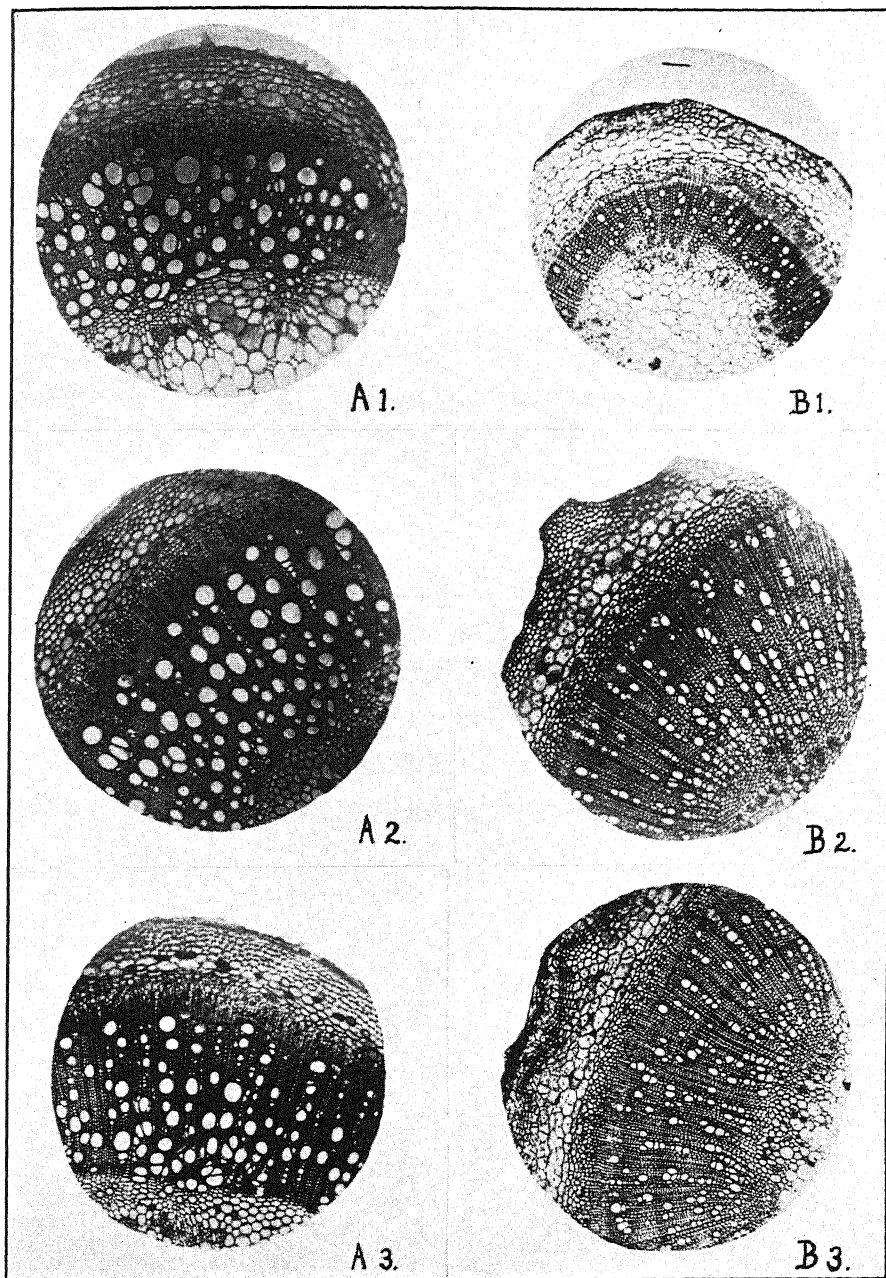
2.



3.

DEATS: PERIOD OF ILLUMINATION





DEATS: PERIOD OF ILLUMINATION

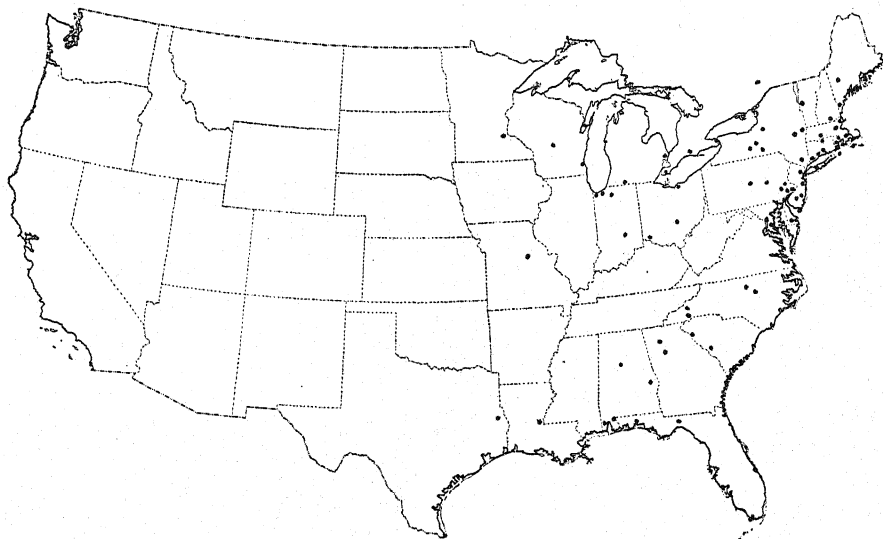


# THE GEOGRAPHICAL DISTRIBUTION OF POISON SUMAC (*RHUS VERNIX* L.) IN NORTH AMERICA

JAMES B. MCNAIR

(Received for publication November 2, 1924)

In the following paper an attempt has been made to determine the geographical distribution of poison sumac (*Rhus Vernix* L.) by means of the locations from which herbarium specimens were taken, correspondence, and locations where birds were found with the seeds of *R. Vernix* in their stomachs. From these locations the plant is found in Canada in Quebec and Ontario, and in the United States in all states east of the Mississippi River with the exception of Kentucky, Tennessee, and West Virginia.<sup>1</sup> It is also found in four states west of the Mississippi, namely, Minnesota, Missouri, Louisiana, and Texas. The habitat of *Rhus Vernix* is in swamps or land inundated at least part of the year.



TEXT FIG. 1. Map showing the geographical distribution of *Rhus Vernix* L. in North America.

The author desires to express his grateful appreciation for the co-operation of M. O. Malte, Department of Mines, Victoria Memorial Museum, Ottawa, Canada; W. C. Henderson, Bureau of Biological Survey, U. S. Department of Agriculture, Washington, D. C.; S. F. Blake, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.;

<sup>1</sup> It is probable that the plant also grows in these states.

W. R. Maxon, U. S. National Herbarium, Washington, D. C.; D. C. Davies, B. E. Dahlgren, Francis Macbride, and Miss Edith M. Vincent, Field Museum of Natural History, Chicago, Ill.; B. L. Robinson, Gray Herbarium, Harvard University, Cambridge, Mass.; Alfred Rehder, Arnold Arboretum, Harvard University, Jamaica Plain, Mass.; J. M. Greenman and Carl Epling, Missouri Botanical Garden, St. Louis, Mo.; W. E. Maneval, University of Missouri, Columbia, Mo.; P. A. Rydberg and Mrs. Palmyra de C. Mitchell, New York Botanical Garden, New York; Francis W. Pennell, Academy of Natural Sciences of Philadelphia, Pa., and to those others listed later in this paper who have corresponded with him.

The letters following the location of the plants indicate the herbaria where the specimens are to be found. These letters and their meanings are: A, Arnold Arboretum; C, Canadian National Herbarium; F, Field Museum of Natural History; G, Gray Herbarium; M, Missouri Botanical Garden; NS, Academy of Natural Sciences; NY, New York Botanical Garden; UM, University of Missouri; US, United States National Herbarium.

#### CANADA

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DISTRICT OF COLUMBIA: Washington, G. McCarthy, F.

FLORIDA: Holton, Chapman, NY; Tallahassee, Rugel, M, NY; Walton County, A. H. Curtiss, NY.

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MISSOURI: Columbia, UM.

NEW HAMPSHIRE: Pelham, Knowlton, G.

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NEW YORK: Danby, Eames and MacDaniels, G; Freeville, —, US; Ithaca, —, US; Ithaca, W. Trelease, M; Long Island, Southampton, St. John, G; Mohegan, Martens, G; Mutton Hill Pond, T. F. Lucy, F; New York, Torrey Herbarium, NY; Oswego, Fernald, Wiegand, and Eames, G; Ovid, Brewer and Chickering, F; Ovid, J. W. Chickering, M; Pennyan, —, M; Poggenburg, J. Park, NY; Prope, J. F. Holton, F; Troy, James Hall, F; West Point, N. Taylor, NY; White Plains, P. A. McCabe, NY.

NORTH CAROLINA: —, A; Biltmore, —, G, M, NY, US; Biltmore, A. A. Heller, F; Chapel Hill, W. W. Ashe, M; Flat Rock, S. B. Buckley, M; Raleigh, Ashe, G.

OHIO: Camden Lake, Lorain Co., US; Cincinnati, C. W. Short, M; Lancaster, —, US.

PENNSYLVANIA: Allentown, Lehigh Co., S. S. Van Pelt, NS; Bangor,

Northampton Co., S. S. Van Pelt, NS; Byberry, Philadelphia Co., F. W. Pennell, NS; Center County, J. T. Rothrock, F; Delaware County, —, US; Dillerville, Heller, G; Dillerville, A. A. Heller, F; Dillerville, J. K. Small, F; Dillerville Swamp, A. A. Heller, NS, NY; Dillerville Swamp, J. K. Small, NY; Elam, Delaware Co., F. W. Pennell, NS; Hillside, Montgomery Co., B. Long, NS; Lancaster County, —, US; Nottingham, Chester Co., B. Long, NS; Pricetown, Berks Co., B. Long, NS; Smithville, J. K. Small, F; Smithville Swamp, J. K. Small, NY; Snyderstown, N. M. Glatfelter, M; Tullytown, A. MacElwee, NS.

RHODE ISLAND: Block Island, Fernald and Long, G; Warwick, —, NY; Warwick, Congdon, G; Warwick, J. W. Congdon, F; Wickford, E. F. Williams, G.

SOUTH CAROLINA: Anderson, —, US; Anderson, L. R. Gibbes, NY; Charleston, L. R. Gibbes, NY; Graniteville, H. Eggert, M; Santee Canal, Ravenel, G.

TEXAS: —, E. J. Palmer, A; San Augustine, —, US.

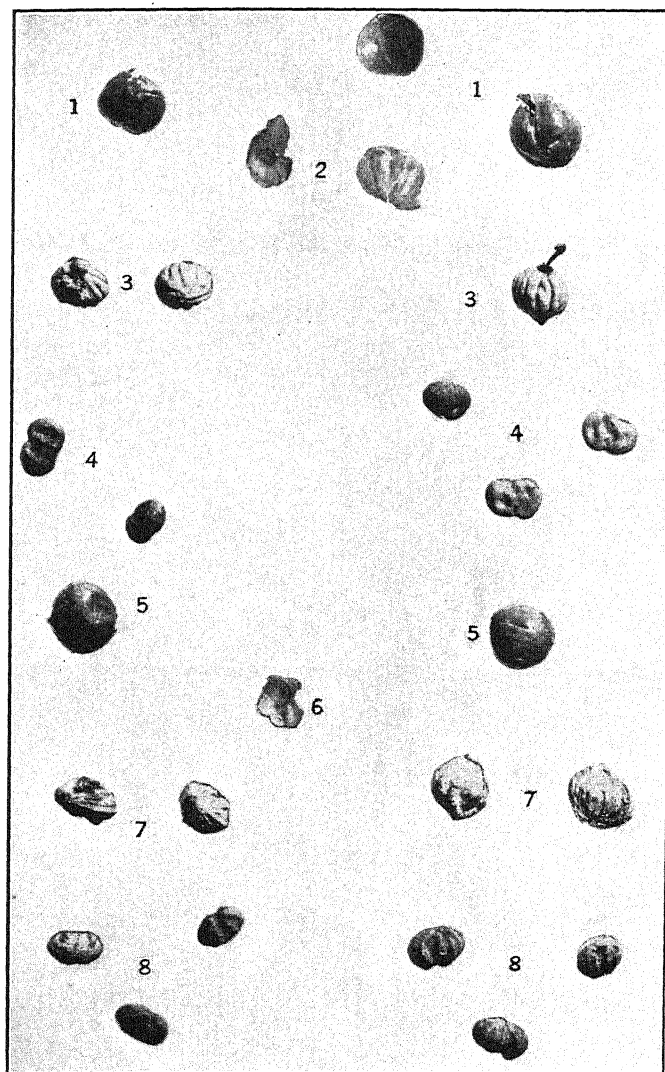
VERMONT: —, C. G. Pringle, A; Middlebury, Brainerd, G.

WISCONSIN: Green Bay, —, US; Green Bay, Schuette, G; Green Bay, J. H. Schuette, NY; Juneau County, —, US; Juneau County, Mearns, G; Milwaukee, I. A. Lapham, M; Milwaukee County, H. E. Hasen, NY.

*R. Vernix* is not known to occur as a native plant in the following states: Arkansas (John T. Buchholz, University of Arkansas, Fayetteville); Colorado (E. C. McCarty, Colorado Agricultural College, Fort Collins); Idaho (Floyd W. Gail, University of Idaho, Moscow); Kansas (L. E. Melchers, Kansas State Agricultural College, Manhattan); Montana (F. B. Cotner, Agricultural Experiment Station, Bozeman); Nebraska (E. R. Walker, University of Nebraska, Lincoln); New Mexico (R. F. Crawford, Agricultural Experiment Station, State College); North Dakota (A. F. Yeager, North Dakota Agricultural College, Agricultural College); Oklahoma (C. O. Chambers, Agricultural and Mechanical College, Stillwater); Wyoming (Aven Nelson, University of Wyoming, Laramie).

W. C. Henderson of the Bureau of Biological Survey, U. S. Department of Agriculture, states that the seeds of *R. Vernix* bear a number of vertical ridges and in this way may be distinguished from the seeds of *R. Toxicodendron*, as these ridges are either entirely absent or greatly obscured in the latter species. A list of localities where birds have been found with the seeds of *R. Vernix* in their stomachs follows:

Alabama: Greensboro. California: Chico, Mt. Veeder. Connecticut: East Hartford, Portland. District of Columbia: Washington. Georgia: Calhoun. Illinois: Cairo, Mound City, Sangamon River, St. Joseph. Maryland: Montgomery County, Silver Springs. Massachusetts: Raynham, Taunton. Kansas: Onaga. New Jersey: Shiloh, Stag Lake, Palisades Park. New York: Amityville, L. I.; Lake George, Warren County;



McNair: DISTRIBUTION OF POISON SUMAC



Shelter Island; Long Island City; Sing Sing. North Carolina: Raleigh. Ohio: Cleveland, Oxford. Ontario: London. Virginia: Dreurys Bluff.

#### EXPLANATION OF PLATE XL

Figures 1 to 4 inclusive are of fruits of *Rhus Toxicodendron* L. from Oklahoma. All are  $1\frac{3}{4} \times$  natural size.

FIG. 1. Views of entire ripe fruit showing its glabrous outer coat (exocarp).

FIG. 2. Portions of the glabrous, readily separable outer coat of the ripe fruit.

FIG. 3. Fruit after the outer coat has been removed, showing the sulcate waxy endocarp.

FIG. 4. Seeds showing their peculiar knobbed protuberances.

Figures 5 to 8 inclusive are of fruits of *Rhus Vernix* L. from the District of Columbia. All  $1\frac{3}{4} \times$  natural size.

FIG. 5. Entire ripe fruit, showing its glabrous appearance.

FIG. 6. Portion of the glabrous outer coat (exocarp) of the ripe fruit.

FIG. 7. Fruit after the outer coat has been removed. The appearance would be similar to that of figure 3 if part of the waxy portion had not fallen off.

FIG. 8. Seeds showing their vertical ridges, markedly distinct in appearance from those shown in figure 4.

## SOME FURTHER EXPERIMENTS ON THE RELATION OF LIGHT TO GROWTH

J. ADAMS

(Received for publication November 24, 1924)

In a previous publication (1) I stated that "in summer the higher slopes of mountains and places in higher latitudes have a longer period of daylight, and this greater duration of daylight even with a lower temperature may produce as great an effect on plants as a shorter period of light with a higher temperature in places lying near the equator." In another and later publication (3) the following statement occurs: "From the experiments above described and from many others the conclusion is drawn that light and heat are to a certain extent interchangeable in a plant's economy." The experiments of which the details follow were undertaken mainly with a view to confirming, or, at any rate, supplying some further information bearing on, the above statements. At the same time some additional tests were made of the value of electrical illumination as a substitute for or an addition to ordinary daylight; finally an experiment was made out of doors to determine the effect on plant growth of the interposition of a screen of ordinary window glass. The details of the experiments are given in the following order: (1) experiments with electric light; (2) experiments on duration and intensity of light; (3) experiments on relation of growth to duration of light and temperature; (4) effect on growth of a screen of colorless glass. Discussion of the results obtained under each of these four heads follows farther on.

### EXPERIMENTS WITH ELECTRIC LIGHT

The plants in this set were divided into three groups. The first set was grown in a cellar under artificial illumination only, daylight being entirely excluded; the second set was grown in a greenhouse and received the natural daily period of daylight only; the third set was also grown in a greenhouse and received the full daily amount of daylight supplemented by electrical illumination at night. In each of these three groups of experiments there were as a rule three pots which were planted with seeds of the same species, the final figures being based on the average of the three. The experiments were started on November 15, 1923.

In the case of the plants grown in the cellar the pots were placed on the floor, the top of the pot being about five feet from the light. The lights in the cellar burned continuously throughout the experiment, except for an interval of 5 hours and for three other short intervals when it was neces-

sary to turn off the current in order to change the bulbs, etc. At first a nitrogen-filled lamp of 300 watts (voltage 110) was used. This was replaced on November 30 by a 500-watt lamp with Trutint Daylight shade, but the Trutint shade was removed on December 7 as it was found that it cut off too much light. On December 20 an additional 200-watt lamp was installed, thus bringing the total up to 700 watts.

In the greenhouse the electric light was turned on every night during the course of the experiment, with the exception of two nights on which it was forgotten. A lamp of 300 watts was used from November 15, 1923, to February 1, 1924, after which date a lamp of 500 watts was employed until March 24 when the artificial illumination at night was discontinued.

The mean temperature in the cellar from November 15, 1923, to March 24, 1924, was 66.8° F., while the extremes varied from 56° F. to 78° F. In the greenhouse during the same period the mean temperature was 57.9° F., while the extremes were 36° F. and 80° F.

The species experimented with under electric light alone comprised wheat, Indian corn, tulip, hyacinth, buckwheat, hemp, flax, castor oil bean, sugar maple, black currant, alfalfa, wax bean, soybean, potato, and sunflower. Of these, wheat, buckwheat, hemp, castor oil bean, wax bean, black currant, potato, and sunflower made much more rapid growth at first than the same species in the greenhouse and developed the natural green color. Only four species, namely, wax bean, castor oil bean, tulip, and hyacinth reached the flowering stage, the flowers in each case being of the normal color. These colors were white in wax bean, yellow in tulip, blue in hyacinth, and red (stigmas) in castor oil bean. The castor oil bean alone developed fruits and ripe seeds. Ten of these seeds were afterwards placed in a pot in the greenhouse, and all germinated and eventually produced vigorous plants when set out in the open ground. The sugar maple evidently requires a long resting period, as the first symptoms of swelling in the buds appeared only on March 24, 1924. In all the other species experimented with, apparently an illumination of 700 watts was insufficient for full development.

In the case of the two sets of plants in the greenhouse the conditions were practically the same in each except in the amount of illumination. The particulars relating to each species were as detailed below.

*Wheat.* The average height on November 26, 1923, of the electrically illuminated plants was 67.6 mm., while that of those exposed to daylight only was 47.3 mm. On March 24, 1924, the average heights of the same two sets were 991.1 mm. and 1209.3 mm., while the average number of ears produced was 3.2 and 2.4 respectively. The dates of coming into ear in the first set were February 9, February 11, and February 9, and in the latter set March 15, March 11, and March 10.

*Buckwheat.* The average height of the electrically illuminated plants on November 29, 1923, was 48.9 mm., while that of those exposed to day-

light only was 34.1 mm. The corresponding heights on December 29, 1923, were 164.0 mm. and 139.0 mm., representing increases of 235.3 percent and 307.6 percent respectively.

*Hemp.* The average height of the electrically illuminated set on November 26, 1923, was 40.5 mm., while that of the set exposed to daylight only was 42.5 mm. On March 22, 1924, the corresponding heights were 898.3 mm. and 896.4 mm. respectively. As regards the date of flowering, the three sets exposed to artificial illumination came into flower on April 3, April 4, and April 4, while in the other set the dates were March 24, March 26, and April 1.

*Wax Bean.* The average height on December 13, 1923, of the electrically illuminated plants was 88.1 mm., while that of the other set was 61.9 mm. The corresponding figures for February 4, 1924, were 184.0 mm. and 125.8 mm., representing increases of 108.8 and 103.2 percent respectively.

*Soybean.* The average height on December 10, 1923, of the electrically illuminated plants was 81.1 mm., while that of the set exposed to daylight only was 39.8 mm. On March 10, 1924, the corresponding figures were 460.7 mm. and 305.0 mm., representing increases of 468.0 and 666.3 percent respectively. Up to May 26, 1924, only flowers of a very rudimentary kind which did not open were produced by all the plants of both sets.

*Potato.* The potato tuber evidently requires a long resting period, as the first sprouts in both the electrically illuminated and the daylight sets were just coming through the surface of the soil on February 1, 1924. The average height of the former on March 21, 1924, was 316.7 mm., while that of the plants exposed to daylight only was 233.3 mm. Up to April 10, 1924, no flowers had developed in either set.

*Sunflower.* The average height of the electrically illuminated plants on November 29, 1923, was 13.0 mm., while that of the set exposed to daylight only was 13.7 mm. On March 17, 1924, the corresponding figures were 890.5 mm. and 853.7 mm. respectively. The electrically illuminated plants came into flower on May 7, May 20, and May 26, while in the set exposed to daylight only the dates were May 12, May 26, and May 26. On May 26, 1924, there were three in flower in the former set, while in the daylight set there were five plants in flower.

*Sugar Maple.* Out of three plants exposed to electric light at night two had come into leaf on March 24, 1924, while the third was still dormant. One of the plants exposed to daylight only was still dormant on the same date, in another the buds were swelling, while the third had come into leaf.

*Black Currant.* Under electrical illumination at night sprouting commenced on December 11, 1923, while under daylight only sprouting was deferred until January 9, 1924. The average length of new growth in the former on March 21, 1924, was 397.5 mm., while in the latter the average length was 225.0 mm. On April 30, 1924, in both cases the new growth was becoming woody and buds for next season's growth were forming.



## EXPERIMENTS ON DURATION AND INTENSITY OF LIGHT

The object of this set of experiments was to compare the photosynthetic effect of sunlight during the midday hours with that in the morning and in the afternoon. For this purpose, one set of plants was covered so as to exclude the light at the same time that the other set was left uncovered. The actual time of exposure naturally varied with the length of the day from sunrise to sunset, but for every hour of midday light received by one set the other set received  $1\frac{1}{2}$  hours, partly in the morning and partly in the afternoon. The covers used were the same as those employed in previous experiments, namely, a light wooden framework covered with sheets of brown paper.

## First Series

This set of experiments was carried out in a greenhouse, the plants used being spring wheat, flax, soybean, and sunflower. There were six pots of each, three being for midday exposure and three for exposure during the morning and afternoon. The seeds were sown on November 21, 1923, and the period of covering the plants extended from November 26, 1923, to March 22, 1924. Altogether the covering and uncovering of the plants took place on 71 days. On certain days both sets of plants were left uncovered in order to maintain healthy growth.

*Wheat.* On December 3, 1923, the average height (measured to the tip of the leaf) of the lot exposed during the middle of the day was 80.0 mm., while that of the other lot was 65.0 mm. On January 2, 1924, the height of the midday lot was 271.0 mm., while that of the other lot was 277.9 mm. On February 6, 1924, the corresponding figures were 389.1 mm. and 352.1 mm. The final measurement on March 6, 1924, gave an average of 286.8 mm. for the midday lot and 199.5 mm. for the other lot, the measurements being made to the top of the uppermost leaf-sheath. The respective increases were 258.5 and 206.9 percent.

The dates of coming into ear of the three midday lots were April 22, May 2, and May 2, while the dates for the other three lots were April 22, May 3, and May 10. The number of ears produced up to May 12 was 20 for the midday set and 9 for the other set.

*Flax.* On December 3, 1923, the average height of the plants exposed at midday was 40.7 mm., while that of the other lot was 32.4 mm. On January 2, 1924, the respective heights were 104.8 mm. and 90.8 mm., while on February 7 the heights were 173.2 mm. and 158.1 mm. The last measurements, on March 20, gave a height of 313.2 mm. for the midday lot and 294.5 mm. for the other lot. The respective increases were 669.5 and 808.9 percent.

The dates of coming into flower of the three midday lots were April 17, April 22, and April 28, while the dates in the other three lots were April 19, April 28, and May 2. The total number of plants in flower up to May 2 was 11 in the case of the midday lots and 7 in the other lots.

*Soybean.* The average height on December 12, 1923, of the plants exposed at midday was 45.7 mm., while that of the other set was 42.1 mm. On January 2, 1924, the corresponding figures were 208.4 mm. and 231.1 mm., while on February 7, 1924, the respective heights were 260.2 mm. and 302.1 mm. The last measurements, on March 20, 1924, gave an average of 295.5 mm. for the midday lot and 353.1 mm. for the other lot. The respective increases were 546.6 and 738.7 percent. In none of the plants were any flowers observed, but all of them showed small rudimentary pods.

*Sunflower.* The average height on December 7, 1923, of the plants exposed at midday was 39.8 mm., while that of the other lot was 36.4 mm. On January 2, 1924, the figures were 193.3 mm. and 175.0 mm. respectively, while on February 8, 1924, the corresponding heights were 326.7 mm. and 293.7 mm. The respective increases were 720.8 and 706.8 percent.

### Second Series

The procedure in this set was similar to that in the first series, but most of the experiments were done in duplicate instead of triplicate. Seeds of soybean and castor oil bean were sown in the greenhouse on March 18, 1924, and of flax and hemp on March 19, 1924. The period of covering the plants extended from March 31, 1924, to May 16, 1924.

*Flax.* The average height of the midday sets on March 28, 1924, was 28.4 mm., while that of the other sets was 28.5 mm. On April 14 the corresponding heights were 108.3 mm. and 92.4 mm. The last measurements, on May 12, 1924, gave an average of 302.4 mm. for the midday lots and 267.7 mm. for the other lots. The total weight (fresh) of plants pulled up by the roots on May 12 was 30.250 grams in the case of the midday lots, while the other lots weighed 25.130 grams.

*Hemp.* The average height of the midday lots on March 28, 1924, was 29.5 mm., while that of the other lots was 29.2 mm. On April 15 the corresponding heights were 124.7 mm. and 109.3 mm. The last measurements, on May 14, gave an average of 475.9 mm. for the midday lots and 416.7 mm. for the other lots. The total weight (fresh) of plants cut at the ground level on May 14 was 118.500 grams in the case of the midday lots, while that of the other lots was 75.020 grams.

*Soybean.* The average height of the midday lots on April 10, 1924, was 66.0 mm., while that of the other lots was 38.8 mm. On May 2 the corresponding heights were 140.4 mm. and 107.2 mm. The last measurements, on May 16, gave an average of 228.2 mm. for the midday lots and 194.5 mm. for the other lots. The respective increases were 245.7 and 401.3 percent. The total weight (fresh) on May 16 of plants cut at the ground level was 38.800 grams in the case of the midday lots, while that of the other lots was 29.590 grams.

*Castor Oil Bean.* The average height on April 14, 1924, of the midday lots was 44.0 mm., while that of the other lots was 44.3 mm. On May 2

the corresponding figures were 82.0 mm. and 84.8 mm., while the final measurements, on May 16, gave an average of 142.1 mm. in the case of the midday lots and one of 116.0 mm. for the other lots. The total weight (fresh) of plants cut at the ground level on May 16 was 49.990 grams in the case of the midday lots and 41.340 grams in the case of the other lots.

### Third Series

This series of plants was grown in the open ground. Large wooden boxes were used as covers to which short legs about 6 inches long were attached at the corners, so that when the boxes were placed in position there was a free space below the cover through which the air could circulate. The date of covering extended from June 17 to July 19, 1924, but was not the same in all species. The seeds of each species were sown in two rows on May 17, 1924, one row to be exposed to light during the midday hours and the other during the morning and afternoon. The chief objection to carrying on experiments of this kind in the open ground is that when it rained one row of plants was covered and the other exposed. To compensate for this, the covered row was watered as soon as the time arrived for removing the cover to the other row.

*Buckwheat.* The average height on June 16, 1924, of the midday row was 82.1 mm., while that of the other row was 76.5 mm. On June 23 the respective heights were 182.6 mm. and 171.6 mm. The last measurements, on July 7, gave an average of 533.5 mm. for the midday row and 516.0 mm. for the other row. The respective increases were 549.8 and 574.5 percent. The average weight (fresh) on July 7 of the plants of the midday row, cut at the ground level, was 19.232 grams, while that of the other row was 24.284 grams. On June 26 two plants of the midday row came into flower, while the first plant in the other row came into flower on June 27.

*Soybean.* The average height on June 16, 1924, of the midday row was 54.2 mm., while that of the other row was 44.5 mm. On July 7 the corresponding heights were 183.3 mm. and 150.5 mm. The last measurements, on July 21, gave an average of 294.7 mm. for the midday row and 277.8 mm. for the other row. The respective increases were 443.7 and 524.2 percent. The average weight (fresh) on July 21 of plants pulled up by the root was 11.018 grams in the case of the midday row and 11.333 grams in the case of the other row. On July 21 none were in flower in the midday row, while on the same date two were in flower in the other row.

*White Mustard.* The average height on June 16, 1924, of the midday row was 61.1 mm., while that of the other row was 46.3 mm. The corresponding figures on June 23 were 125.7 mm. and 101.6 mm. The final measurements, on July 12, gave an average of 548.5 mm. for the midday row and 534.1 mm. for the other row. The respective increases were 797.7 and 1053.5 percent. The average weight (fresh) on July 12 of plants pulled up by the roots was 38.119 grams in the case of the midday row and

15.770 grams for the other row. The first flower in the midday row opened on July 8, while in the other row the first flower opened on June 30, and on July 8 seven were in flower.

*Sunflower.* The average height on June 16, 1924, of the midday row was 47.7 mm., while that of the other row was 41.3 mm. On July 8 the corresponding heights were 420.4 mm. and 353.1 mm. The final measurements, on July 17, gave 580.0 mm. for the midday row and 514.1 mm. for the other row. The respective increases were 1115.9 and 1144.7 percent. The average weight (fresh) on July 17 of plants cut at the ground level was 36.203 grams in the case of the midday row, while that of the other row was 31.875 grams. None were in flower in either row.

*Hemp.* The average height on June 16, 1924, of the midday row was 52.5 mm., while that of the other row was 57.5 mm. The corresponding heights on July 8 were 230.4 mm. and 223.5 mm. The last measurements, on July 21, gave an average of 460.1 mm. for the midday row and 431.1 mm. for the other row. The respective increases were 776.3 and 649.7 percent. The average weight (fresh) of plants pulled up by the roots on July 21 was 11.330 grams in the case of the midday row and 12.749 grams in the case of the other row. None had yet come into flower in either row.

#### EXPERIMENTS ON RELATION OF GROWTH TO DURATION OF LIGHT AND TEMPERATURE

##### First Series

Eight species of plants were used for the experiments, which were in duplicate with the exception of castor oil bean and Indian corn, which were in triplicate. The seeds in most of these species were sown on March 18 and 19, 1924. The experiments were carried out in a greenhouse, and daily records were kept of the maximum and minimum temperature and of the time of sunrise and sunset.

*Flax.* The average height on March 27, 1924, was 25.0 mm., while that on May 8 was 319.0 mm. The increase in growth amounted to 1176.0 percent. The number of hours of daylight during this period was 572 hrs. 54 min., and the mean temperature was 60.8° F. The average weight (fresh) of plants pulled up by the roots on May 8 was 1.875 grams.

*Hemp.* The average height on March 27, 1924, was 29.8 mm., while that on May 8 was 844.5 mm. The increase in growth amounted to 2733.9 percent. The number of hours of daylight during this period was 573 hrs. 14 min., and the mean temperature was 60.8° F. The average weight (fresh) of plants cut at the ground level on May 8 was 26.700 grams.

*Soybean.* The average height on April 7, 1924, was 15.2 mm., while that on May 19 was 258.9 mm. The increase in growth amounted to 1603.3 percent. The number of hours of daylight during this period was 593 hrs. 11 min., and the mean temperature was 60.9° F. The average weight (fresh) of plants cut at the ground level on May 19 was 5.854 grams.

*Castor Oil Bean.* The average height on April 2, 1924, was 104.4 mm., while that on May 14 was 454.7 mm. The increase in growth amounted to 335.5 percent. The number of hours of daylight during this period was 583 hrs. 38 min., and the mean temperature was 61.1° F. The average weight (fresh) of plants cut at the ground level on May 14 was 47.664 grams. On this date, out of seven plants altogether, stigmas were protruding in three plants and stamens in one plant.

*Buckwheat.* The average height on March 31, 1924, was 29.4 mm., while that on May 5 was 728.8 mm. The increase in growth amounted to 2378.9 percent. The number of hours of daylight during this period was 478 hrs. 38 min., and the mean temperature was 60.5° F. The average weight (fresh) of plants cut at the ground level on May 5 was 13.002 grams. On May 3 all the plants, ten in number, were in flower.

*Sunflower.* The average height on March 31, 1924, was 23.1 mm., while that on May 12 was 814.8 mm. The increase in growth amounted to 3427.3 percent. The number of hours of daylight during this period was 580 hrs. 49 min., and the mean temperature was 60.9° F. The average weight (fresh) of plants cut at the ground level on May 12 was 47.917 grams.

*White Mustard.* The average height on March 27, 1924, was 23.6 mm., while that on May 8 was 769.7 mm. The increase in growth amounted to 3161.4 percent. The number of hours of daylight during this period was 573 hrs. 19 min., and the mean temperature was 60.8° F. The average weight (fresh) of plants cut at the ground level on May 8 was 31.900 grams.

*Indian Corn.* The average height on April 7, 1924, was 107.0 mm., while that on May 19 was 860.4 mm. The increase in growth amounted to 704.1 percent. The number of hours of daylight during this period was 593 hrs. 36 min., and the mean temperature was 60.9° F. The average weight (fresh) of plants cut at the ground level on May 19 was 40.808 grams.

### Second Series

The first series of experiments extended from March 27 to May 19, while the second set covered the period from May 20 to July 2. As the days were longer during the second period, it was necessary to cover the plants at intervals in order to attain the object of the experiments. The total period of covering the plants extended from May 22 to June 24. The total number of hours of exposure to light is given in connection with each species. The time of sowing varied according to the species, but the dates were mostly between May 2 and May 14. As in the first series the experiments were mostly in duplicate, the exceptions being castor oil bean and Indian corn which were again in triplicate.

*Flax.* The average height on May 20, 1924, was 47.6 mm., while that on July 2 was 395.0 mm. The increase in growth amounted to 729.8 percent. The total time of exposure to daylight was 526 hrs. 47 min., and the mean temperature during this period was 68.3° F. The average weight (fresh) of plants pulled up by the roots on July 2 was 3.203 grams.

*Hemp.* The average height on May 20, 1924, was 61.1 mm., while that on July 2 was 627.7 mm. The increase in growth amounted to 927.3 percent. The total time of exposure to daylight amounted to 527 hrs. 17 min., and the mean temperature during this period was 68.3° F. The average weight (fresh) of plants cut at the ground level on July 2 was 10.047 grams.

*Soybean.* The average height on May 20, 1924, was 30.0 mm., while that on June 30 was 595.5 mm. The increase in growth amounted to 1885.0 percent. The total time of exposure to daylight amounted to 500 hrs. 6 min., and the mean temperature during this period was 68.1° F. The average weight (fresh) of plants cut at the ground level on June 30 was 16.169 grams. Out of a total of ten plants, six were in flower on this date.

*Castor Oil Bean.* The average height on May 21, 1924, was 133.0 mm., while that on July 2 was 562.1 mm. The increase in growth amounted to 322.6 percent. The total time of exposure to daylight amounted to 515 hrs. 26 min., and the mean temperature during this period was 68.4° F. The average weight (fresh) of plants cut at the ground level on July 2 was 68.290 grams. Out of a total of nine plants, stamens and stigmas were protruding in four.

*Buckwheat.* The average height on May 22, 1924, was 13.9 mm., while that on June 26 was 586.6 mm. The increase in growth amounted to 4120.1 percent. The total time of exposure to daylight amounted to 418 hrs. 45 min., and the mean temperature during this period was 67.7° F. The average weight (fresh) of plants cut at the ground level on June 26 was 15.095 grams. Out of a total of ten plants, five were in flower on this date.

*Sunflower.* The average height on May 20, 1924, was 26.4 mm., while that on June 30 was 827.8 mm. The increase in growth amounted to 3035.6 percent. The total time of exposure to daylight amounted to 498 hrs. 56 min., and the mean temperature during this period was 68.1° F. The average weight (fresh) of plants cut at the ground level on June 30 was 30.465 grams.

*White Mustard.* The average height on May 21, 1924, was 16.1 mm., while that on July 2 was 609.3 mm. The increase in growth amounted to 3684.5 percent. The total time of exposure to daylight amounted to 511 hrs. 51 min., and the mean temperature during this period was 68.4° F. The average weight (fresh) of plants cut at the ground level on July 2 was 19.102 grams.

*Indian Corn.* The average height on May 20, 1924, was 85.9 mm., while that on June 30 was 1067.1 mm. The increase in growth amounted to 1142.2 percent. The total time of exposure to daylight amounted to 499 hrs. 56 min., and the mean temperature during this period was 68.1° F. The average weight (fresh) of plants cut at the ground level on June 30 was 66.688 grams.

## EFFECT ON GROWTH OF A SCREEN OF COLORLESS GLASS

In order to determine the influence of a covering of ordinary window glass on the sun's rays while passing through it and the effect on plant growth, the present series of experiments was undertaken. Four species were employed, namely, wheat, buckwheat, white mustard, and flax. The plants were grown out of doors, one set of plants being covered with a large window such as is used for covering a forcing frame. This was supported at the ends at a convenient height above the plants so as to allow air to circulate freely beneath it. The glass covering had the effect of excluding rain from the plants beneath it, but to compensate for this the covering was removed at intervals and the plants were watered with tap water. In order to determine whether the temperature under the glass was the same as that outside it, three tests were made. On July 3 the temperature in the sun under the glass at 9:45 A.M. was 105° F., while that outside it at the same time was 112° F. On July 4 the sun temperatures at 9:15 A.M. were 88° F. under the glass and 96° F. outside it. On July 15 the temperature in the sun under the glass at 10:00 A.M. was 103° F., while that outside it was 106° F.

The seeds of both lots were sown on June 11, 1924. The glass window was placed in position on June 25, and remained until the close of the experiment except when it was removed for watering.

*Wheat.* There were 12 plants in each lot. The average height of the plants under glass was 126.5 mm. on June 24; 207.7 mm. on July 4; 274.7 mm. on July 15; 324.6 mm. on July 22. The corresponding heights on the same dates of the plants in the open were 125.7 mm.; 194.6 mm.; 252.6 mm.; 285.5 mm. The average weight (fresh) on July 22 of plants pulled up by the roots was 3.733 grams in the case of the plants under glass and 4.546 grams in the case of the plants grown in the open.

*Buckwheat.* There were 12 plants in each lot. The average height of the plants grown under glass was 34.8 mm. on June 24; 113.9 mm. on July 4; 371.5 mm. on July 11. The corresponding heights on the same dates of the plants in the open were 37.5 mm.; 111.0 mm.; 388.2 mm. The average weight (fresh) on July 11 of plants cut at the ground level was 17.092 grams in the case of the plants under glass and 19.017 grams in the case of the plants grown in the open. There were none in flower under glass, while one of the plants in the open had come into flower.

*White Mustard.* There were 12 plants in each lot. The average height of the plants grown under glass was 26.0 mm. on June 25; 89.0 mm. on July 3; 343.1 mm. on July 11. The corresponding heights on the same dates of the plants in the open were 25.2 mm.; 71.2 mm.; 272.8 mm. The average weight (fresh) on July 11 of plants pulled up by the roots was 22.558 grams in the case of the plants grown under glass, while that of the plants grown in the open was 20.383 grams.

*Flax.* There were 20 plants in each lot. The average height of the plants grown under glass was 37.8 mm. on June 25; 94.9 mm. on July 3; 260.4 mm. on July 15; 345.6 mm. on July 22. The corresponding heights on the same dates of the plants in the open were 36.0 mm.; 83.2 mm.; 216.2 mm.; 318.2 mm. The average weight (fresh) on July 22 of plants pulled up by the roots was 5.697 grams in the case of the plants grown under glass, while that of the plants grown in the open was 5.597 grams.

## DISCUSSION OF RESULTS

### Experiments with Electric Light

The results obtained under electric light alone confirm those of Hendricks and Harvey (6) and show that many species of plants can carry on their life processes without daylight provided that the intensity of illumination is sufficient. The natural color of leaves and flowers was also developed under electrical illumination. As in the case of daylight, the requirements of different species in regard to light intensity vary very considerably under electrical illumination. Whether, as seems probable, some plants would show better results under alternation of periods of electric light and darkness has not been determined. It is worthy of note in this connection that Hendricks and Harvey (6) found that, while flower buds were formed under continuous illumination in *Hibiscus Trionum*, they refused to open until the lights had been turned off for an hour. The dormant habit in sugar maple must be very deep-seated, as neither the stimulus of light nor the effect of a sufficiently high temperature was able to induce the first signs of growth much in advance of the natural period of plants growing out of doors. In wheat, buckwheat, and soybean, the plants exposed to daylight alone were taller than those under daylight plus electrical illumination; while in hemp, wax bean, and sunflower, the addition of electric light resulted in a slight increase in growth as compared with the daylight group. Under electrical illumination at night, earlier flowering occurred in wheat and sunflower and later flowering in hemp.

As in some previous experiments (2) the use of electric light in certain species appears to be of doubtful value.

### Experiments on Duration and Intensity of Light

It is apparent from these experiments and from those carried on by others (5, 11) that studies of the effect on plant growth of shortening or lengthening the time of daily exposure to light lead to no very definite conclusions unless at the same time some observations have been made on the intensity of the light during the time of exposure. Koningsberger (7) claims that the light intensity is the limiting factor rather than the duration of illumination. Stålfelt (9), experimenting with leaves of pine and spruce under natural conditions, found that the rate of assimilation fell off very



markedly in older leaves at the higher light intensities, but that in light intensities of about 30 percent of full sunlight leaves five years of age absorbed much more carbon dioxid per hour than did leaves one year old.

In the experiments outlined above, as proper instruments for measuring the intensity of light were not available, some of the plants were exposed to the brighter light of midday while others were exposed to the duller light of the morning and afternoon, the time of exposure in the two cases being in the proportion of 2 to 3.

In the first series, wheat and sunflower made greater growth during the midday hours while the reverse was the case in flax and soybean.

In the second series, flax, hemp, and castor oil bean made greater growth under midday exposure while this condition was reversed in the case of soybean.

In the third series, hemp made better growth during the midday hours while the reverse was true in the case of buckwheat, soybean, white mustard, and sunflower.

Altogether, better growth was made during midday in six cases while the reverse was true in seven cases.

As regards the time of production of flowers, in those species which bloomed it was observed that the flowers opened first in the midday groups in three cases while the reverse was true in two cases.

At the close of the experiment the fresh weight was taken of the plants in the second and third series only. Taking into account the difference in height at the time when the experiment started, it was found that in four cases the weight of the midday lots was greater while the reverse was true in three cases. In sunflower and hemp the weights were about the same under each exposure to light. In every experiment soybean gave a better result under the more subdued light.

From these experiments the conclusion seems warranted that 4 or 6 hours of light in the middle of the day have the same effect on plant development as 6 or 9 hours divided between the morning and the afternoon.

#### Experiments on Relation of Growth to Duration of Light and Temperature

The results obtained by Eaton (4), who kept the light factor constant but varied the nightly temperature, are of interest in this connection. He subjected three sets of Peking soybeans to a thirteen-hour day out of doors. During the nights one set of these was placed in a chamber with a temperature of about 50° F., the second set in a control chamber at about 65°, and the third was given a nightly temperature of 90°. The differences in the time of flowering of these sets of plants were almost as great as the differences effected by Garner and Allard by exposing plants to varied lengths of day. The set given the cold nights flowered on the forty-fifth day after germination, those in the check chamber on the twenty-fifth day,

and those in the hot-night chamber on the twenty-first day. He obtained similar results with phlox and aster.

In the two series of experiments of which the particulars were given above, the first set of plants received a longer exposure to daylight but the temperature was lower. In the first series, a greater increase in growth was shown in flax, hemp, castor oil bean, and sunflower as compared with the second series, whereas in the second series soybean, buckwheat, white mustard, and Indian corn showed greater height as compared with the corresponding species in the first series.

As regards the fresh weight of the plants, the average was higher in the first series in hemp, sunflower, and white mustard, while in the second series the average was higher in flax, soybean, castor oil bean, buckwheat, and Indian corn. The averages in the two sets, taking all the eight species into account, are as follows:

*First Series.* Average height at beginning, 44.7 mm.; average increase, 1940.0 percent; average duration of daylight, 568 hrs. 40 min.; average temperature, 60.8° F.; average weight, 26.965 grams.

*Second Series.* The corresponding figures were: height, 51.7 mm.; increase, 1980.9 percent; daylight, 499 hrs. 53 min.; temperature, 68.2° F.; weight, 28.632 grams.

In the first series the duration of daylight was 13.7 percent more than in the second, while in the second series the temperature was 12.2 percent higher than in the first series.

As might be expected, the average weight of the plants in the second series was somewhat greater than that of the first series, allowing for the fact that the plants of the second series were taller at the beginning of the experiment.

From these results it seems highly probable that the daylight factor plays an important part in determining the north-and-south range of many plants which exist in localities as far apart as Newfoundland and Georgia.

### Effect on Growth of a Screen of Colorless Glass

A comparison of the figures obtained in the experiments carried out under this head shows that the intervention of a sheet of clear glass had no appreciable effect on the development of the plants growing beneath it when compared with other plants growing in the open. In the account of his experiments on the movements of stomata, Loftfield (8) says:

Fully 50 percent of the light is removed in a greenhouse with clean panes. In the case of panes only lightly frosted it is probable that less than 15 percent of the light incident actually reached the plants.

In the greenhouse at the University of Minnesota, owing to the remains of a coat of whitewash and to soot from the trains passing just below, only 17 percent of the light outside penetrated through the glass. This light was a diffused sunlight and much more than could pass through a canvas covering, such as used by Gray and Peirce in their experiments, or through a north window, such as used by Darwin.

Loftfield further states in the same publication that "stomata open at night as a result of moonlight or a strong artificial light of much less intensity than 1 percent of the sunlight maximum." Stålfelt (9) states that in conifers there is little or no assimilation below about 5 percent of full sunlight.

In a review of recent investigations on carbon assimilation, Stiles (10) mentions some other experiments of Stålfelt on sun and shade leaves. Using different intensities of illumination, Stålfelt found that "with increasing illumination the rate of assimilation increases, so that the maximum rate of assimilation in the open air is only reached with full sunlight." Lundegårdh (10) as the result of his studies on certain forest plants and shore plants states that "when the carbon dioxide concentration was that in normal air, it was found that with increasing light intensity the assimilation by shade plants increased more or less in proportion to the intensity of the light up to an intensity of illumination of about one-tenth of full sunlight, above which intensity of illumination no further increase in assimilation took place." Boysen Jensen (10) concludes "that the maximum assimilation of plants growing under natural conditions is exhibited in light intensities well below that of full sunlight, the maximum assimilation of forest trees being reached in an intensity of illumination of 5.35 percent that of full daylight."

It would be rather hopeless at present to reconcile such divergent views. If only 50 percent of the light is able to pass through the glass of a greenhouse, as stated by Loftfield, one would expect a quite different result from that found by me in experiments along this line. The view of Boysen Jensen, that only a little over 5 percent of sunlight is effective and the remaining 95 percent of no avail in promoting assimilation, does not seem reasonable seeing that light of some kind is one of the prime necessities for plant growth.

#### SUMMARY

Under a continuous electrical illumination of 700 watts (voltage 110) with daylight entirely excluded, castor oil bean completed its life history from seed to seed and produced vigorous plants from the seeds ripened under electric light.

Flowers developed their natural colors under the same illumination, namely, white in wax bean, yellow in tulip, blue in hyacinth, and red (stigmas) in castor oil bean.

Plants of wheat, buckwheat, and soybean exposed to daylight alone made greater growth than those exposed to daylight plus electrical illumination at night, while hemp, wax bean, and sunflower showed a slight increase as the result of the nightly illumination. Under the additional illumination at night, earlier flowering occurred in wheat and sunflower and later flowering in hemp.

Observations as regards height, time of flowering, and weight made on wheat, flax, hemp, buckwheat, white mustard, soybean, castor oil bean, and sunflower grown both in a greenhouse and in the open air showed that the results were as satisfactory under two hours' exposure to light at midday as under three hours' exposure during the morning or afternoon.

Experiments in a greenhouse with Indian corn, flax, hemp, castor oil bean, buckwheat, white mustard, soybean, and sunflower carried out at different times of the year showed as good a growth under an average exposure to daylight for 568 hrs. 40 min. at a mean temperature of 60.8° F. as they did with an average exposure to light of 499 hrs. 53 min. at a mean temperature of 68.2° F.

Experiments in the open air with wheat, buckwheat, white mustard, and flax showed as vigorous growth under a glass screen as that of a corresponding set in the absence of such a screen.

The conclusion is drawn that experiments on the relation of plants to light, in order to be of value, must take into account not only the duration of light but also measurements of its intensity, as well as records of the temperature throughout the period of the experiments.

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CENTRAL EXPERIMENTAL FARM,  
OTTAWA

# THE CONDITIONS OF INFECTION IN POTATO WART

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## INTRODUCTION

*Synchytrium endobioticum* is an obligate parasite. The only phase of its life cycle which is independent of a living host is the germination of the resting and soral sporangia and the ensuing swarm period of the zoöspores, which ordinarily is not prolonged beyond an hour or two. The investigation of the extrinsic factors of infection is therefore limited to an amplitude within which the host is able to exist, and can not be supplemented by a study of the pathogen in culture, such as in many other plant diseases so largely contributes to an understanding of infection and parasitism. Aside from the scientific interest, however, the practical importance of determining the environmental and other conditions under which potato wart develops<sup>1</sup> assumed some magnitude when this disease was discovered in the United States in 1918 (22, 37).

In particular it became desirable to know whether such distinct differences in susceptibility as had been discovered among British potato varieties also occurred in American varieties, and whether climate would play so definitely restrictive a rôle in the potential spread and severity of the disease as had been determined for powdery scab caused by *Spongospora subterranea* (27). The studies here reported were carried out mainly in the wart-infested area near Freeland, in eastern Pennsylvania, supplemented by experiments in apparatus for the control of environmental factors at Washington, D. C., and State College, Pennsylvania. The reaction of American potato varieties and other hosts to wart has been determined (54). From observations of four years on the control of wart in Pennsylvania by the enforced planting of immune varieties, the conclusion was reached (51) that under our conditions this appears to be only a minor disease susceptible of effective control. This conclusion rests primarily on its controllability and on the absence, under quarantine conditions, of an effective means of rapid spread; in relation to its host, the potato, *S. endobioticum* is found to be a high type of parasite, in general dependent on external conditions favorable to the host.

## THE INTRINSIC FACTORS OF INFECTION

### The Host Range

In biological interest and practical importance the white potato (*Solanum tuberosum* L.) is the principal host. Cotton (9) found that *Solanum nigrum*

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*L.* and *S. dulcamara* L. developed wart infections in pot experiments, though neither had been observed to be attacked in nature. Massee's statement (24) that beets and mangels also are susceptible resulted from a confusion of this *Synchytrium* with *Urophlyctis leproides* Trabut (Mag.), which causes a root gall of beets. In 1919 Kunkel and Orton (22) discovered that a number of varieties of tomato (*Lycopersicon esculentum* Mill.) became infected with wart when grown in naturally infested soil.

In extensive tests of a number of species belonging to the Solanaceae (54) the susceptibility of the three species above named was confirmed, but no additional hosts were found. Subsequently, three additional species of *Solanum*—*S. Jamesii*, *S. Commersonii*, and *S. chacoense*, the first a native of southwestern United States, the other two of South American origin—developed wart in naturally infested soil, but additional tests with species of *Physalis*, *Capsicum*, and *Cyphomandra* proved negative.

### Varietal Difference in Susceptibility

The recognition of immune varieties first grew out of the observation by growers in England that certain kinds of potatoes never became infected although grown in soil in which other sorts were a total loss from the disease (13, 23, 49). This was confirmed by systematic tests of all obtainable British varieties (47, 48), and similar variety tests have been carried out in all the principal countries where the disease occurs (2, 42-45, 56).

In the United States these tests have demonstrated the existence of about 34 immune varieties, some of which are, however, synonyms. Although it appeared at first that no correlation exists between immunity and horticultural type, more extensive evidence has shown that varieties which may be assembled into more or less natural groups react similarly to this disease (52). No correlation between immunity and any specific character, as season of maturity or sprout and tuber color, has been established. In general, the Irish Cobbler, Spaulding Rose, McCormick, Burbank, and Green Mountain *types* are immune, whereas the Triumph, Early Rose, Early Ohio, Rural, Pearl, American Giant, and Up-to-date *types* are susceptible.

Immunity to wart appears to be as constant in potato varieties as any horticultural character, and has been shown to be heritable in a definite manner (35, 55). The nature of this immunity has been the object of experimental attack (6, 33) but still remains unknown. No macroscopically visible response occurs when an immune potato is inoculated in a way that produces marked effects in a susceptible variety. The presence of a particular variety is therefore indispensable to infection of the potato, but no variety of tomato has yet been found to be immune.

### Parts of the Host Attacked

All parts of the shoot system of the potato may be infected, the root system only being immune (6). Schilberszky's original description (39) was founded on infected tubers. Borthwick (5) described and illustrated infection of the stolons, stem base, and leaves. Gough (13) mentioned infection of a potato inflorescence, and Schneider (40) found a resting sporangium in the base of an epidermal hair. The magnitude of the overgrowths is sometimes considerable, depending on the part of the host attacked and its stage of development. Infection of stolon buds and tuber eyes is most frequent and results in the largest tumors, but under favorable conditions shoots and foliage leaves may be severely infected and develop a more massive tissue than corresponding normal parts.

No authentic instance of root infection of the potato has been recorded, though Johnson (17), Bally (4), and others believed that this organ could be attacked. This may have resulted from a misapprehension as to the identity of the parasite or of the infected tissue. *Spongospora subterranea* and the root-knot nematode cause malformation of potato roots suggestive of overgrowth due to wart, and it frequently happens that stem tissue immediately adjacent to a root becomes infected by *Synchytrium*, the resulting proliferation more or less enveloping the root, but there is no tissue connection between the two. Efforts to infect roots artificially have been reported by Malthouse (23), and have been made in the present study. Fresh young roots in active growth were inoculated with germinating sporangia of both kinds, but no evidence of infection was seen, though the same inoculum was effective when applied to shoots. Potato plants in solution cultures were inoculated when a number of stolons, young tubers, and fresh roots were present. In all cases the selective infection of the shoot system was absolute (fig. B, Pl. XLI). Infection sometimes occurred of cells lying in the cortex through which roots were making their outward passage, such infected cells being separated by not more than a cell or two from those belonging to the root (fig. A, Pl. XLI).

It has been stated that infection of both root and shoot systems may occur in the tomato, bittersweet, and nightshade (6). Kunkel and Orton reported wart infection of tomato roots. Cotton (9) observed infection at the base of the stem of *Solanum nigrum*, whereas in *S. dulcamara* spores of *Synchytrium* were present in the stem cortex in the absence of hypertrophy.

In tests with tomatoes, in which upwards of a thousand plants have been examined and several hundred found infected, nothing indicative of root infection has been observed. Attempts to infect tomato roots in solution cultures by methods similar to those used with potatoes were successful only in producing infection on adventitious stem-buds and leaves.

Rarely in nature are tuber infections apart from the eyes found, but sometimes, in moist soil, penetration of the periderm apparently occurs, resulting in the production of a scab-like crust of hypertrophied tissue

(fig. A, Pl. XLIII). In experimental inoculations infection of stem internodes and leaf blades may be brought about; on stems and leaf veins the response is the production of small leaf-like excrescences, but on the inter-venal surface only epidermal hypertrophies characteristic of other *Synchytrium* infections result (figs. B, C, Pl. XLIII).

### The Parasite

To Percival (30) we owe the first comprehensive account of the life cycle of *Synchytrium endobioticum*, but Curtis (10) furnished many additional details, and in particular explained the origin of the sorus of sporangia and the alternative development of zoöspores into sori or resting sporangia.

In brief, the life cycle consists, beginning with a zoöspore which has gained entrance into a host cell, of a cyst or prosorus which enlarges until the greater part of the cell is occupied. Still in a uninucleate condition, it migrates through a pore, prepared in its outer membrane, distally into the host cell. The nucleus then undergoes repeated division, and a new membrane is formed about the entire protoplast; within this membrane the protoplasm segments into 3-7 thin-walled sporangia, the whole constituting the sorus. By further nuclear divisions zoöspores are finally formed. Rupture of the soral envelope and host cell wall then ensues, and ultimately the sporangia set free a large number of motile cells. These may function either as zoöspores or as gametes. If the former, the life cycle is repeated when a congenial host is penetrated; or they may fuse in pairs, forming zygotes which penetrate in the same manner as zoöspores, but, once within the host cell, enlarge to form thick-walled resting spores. The latter after a dormant period germinate by the direct production of zoöspores.

The name to be applied to the potato-wart fungus has been a matter of some contention. Originally described as a new genus—*Chrysophlyctis*—it was transferred to *Synchytrium* by Percival. Curtis showed that in all essential features it has a counterpart among unquestioned species of *Synchytrium*. Following Percival's change of nomenclature, some question arose as to its validity. Although these objections were doubtless considered by Curtis, she does not specifically refer to them. For the sake of clearing up this point in the literature it may not be amiss to review the pertinent facts.

Although Percival observed groups of sporangia which he termed sori, he does not state, nor do his figures show, that the sporangia are formed within a common cell wall. He states that they "are thin transparent sacs either produced singly or 2 to 5 together, forming a sorus of sporangia inclosed within a brown coat which consists largely of the brown altered cell walls of the host. . . ." Schröter (41), however, limits *Synchytrium* to forms whose sporangia are surrounded by a common cell membrane, that of the mother cell. Horne (14) raised this question, and that of the manner of germination of the resting sporangia of the wart fungus, assuming that the latter occurs, as Massee (25) described it, by the extrusion of a thin-walled sac from which the zoöspores then escaped. Bally (4)



argued for the retention of *Chrysophlyctis* on the grounds that mitosis of the primary nucleus is unknown and that amitotic divisions predominate, also that it is distributed within the host tissue by host-cell divisions—a feature unknown in other *Synchytriaceae*. Lindau (21) doubtfully referred it to the *Olpidiaceae*, and was followed by von Minden (28), a disposition admittedly on incomplete information.

The fact that the sorus of sporangia is formed by segmentation within the sorus mother cell is well shown in Curtis' figures 60 and 72a. Köhler's (20) figure 9 also shows this condition. The soral envelope is very delicate and easily ruptured, but may be seen in living material (Pl. XLII). In the mature resting sporangium the zoöspores are inclosed within a 3-layered membrane, the inner layer of which is thin and hyaline. According to Curtis, this membrane functions in the rupture of the outer membranes in germination, after which it lies in folds within the empty spore case. Sometimes, however, in drops of water it may float out so as to appear like a sac extruded from the sporangium. The outermost membrane, deposited from the disorganized remains of the host-cell contents, is brittle and may be fractured so as to liberate the sporangium within. Either process imperfectly simulates the extrusion of a spore-containing vesicle, possibly thus occasioning the belief that germination occurred in this way.

Tobler (50), though accepting *Synchytrium*, would assign *S. endobioticum* to a position somewhat removed from other members of the genus because it is unique among the *Synchytriaceae* in causing active division of the host cell, resulting in large and complex overgrowths. This is possibly a property of the host or of the tissue invaded, not uniquely a character of the parasite. *Synchytrium* species are predominantly parasites of leaf and shoot, in which they cause only local malformations. No other species characteristically attacks storage organs. On hosts other than the potato the malformations induced by *S. endobioticum* are not extensive, and even in the potato, when relatively permanent tissues as in the stem internode or leaf are infected, the resulting hypertrophy is only comparable with that induced in other plants by other species of the genus. This fungus is therefore closely bound to typical members of the genus in both its cytomorphology and its biology.

No evidence of biologically different strains of *S. endobioticum* exists. The behavior of the parasite has been consistent in respect to the varieties of potato which it attacks in all its centers of distribution, and in tests where potato varieties have been exchanged between the United States, the British Isles, and Continental Europe.

### Presence of the Parasite in Virulent Form

The interval between successive generations of the soral sporangia is about 10 to 12 days, and at maturity germination may occur within three hours after exposure to suitable conditions. Zoöspores derived from soral sporangia may therefore be present in an infected potato planting closely following each rainy period during the growing season.

The resting sporangia undergo a dormant period, which in nature is about 8 months, before germination occurs. By experimental control of the environment Curtis (10) induced resting sporangia to germinate in 10 weeks after they were mature, and Brierley (6) found that by suspending them in water about 10 percent germinated in 20 days, and 30 percent germinated in the same time in dilute potato juice. Freezing did not accelerate germination. In the present investigation no advantage has been detected in submitting the resting spore material either to freezing or

to a chilling period before employing it in inoculation experiments. Spores collected in the field in September and stored indoors gave rise to infection in the greenhouse by December; and in one instance a tumor produced in the greenhouse resulted, under conditions which precluded the activity of soral sporangia, in new infection in eight weeks.<sup>2</sup> It is possible that in nature resting sporangia may germinate the same season that they are formed, but a large proportion overwinter, as an examination of fresh or disintegrating warts always discloses large numbers of resting spores which have become dormant before the differentiation of zoöspores.

Germination of both types of sporangia occurs in water alone. Whether dilute potato extract exerts a chemotactic influence is unknown, but Curtis found that germination of resting sporangia was more rapid in rain than in soil water or potato extract. On the other hand, she observed that zoöspores tended to congregate adjacent to meristematic tissue; thus it seems likely that in nature a chemotactic influence originating in the host may actually be operative as a special condition for infection. Esmarck (11) found that soil water exerts a favorable influence on germination of resting spores.

Germination of the resting sporangia is so profoundly influenced by the rest period that a definite period for the process hardly can be stated. Johnson (17) first observed germination in sporangia kept for 3 to 5 days in potato extract, and Percival first found empty sporangia in water after 5 to 6 days. Curtis states only that the zoöspores are usually active for an hour or two before their liberation. In my germination cultures, empty sporangia were observed after an interval of two days, and the proportion increased during the succeeding 5 to 10 days. All the sporangia did not germinate during the longest period that the mounts could be kept free from contamination.

Ordinarily the swarm period does not last more than 20 to 40 minutes, though Curtis found that it may be prolonged for 2 hours at a temperature below 15° C. The zoöspores are probably incapable of independent movement for more than a few millimeters during this brief swarming period. Carriage by water currents may increase their range, as indicated by a statement in Leaflet 105 of the Board of Agriculture, London (1), that the maximum spread of infection on level ground is about 9 inches a year, and down a slope it is about 30 inches. In experiments to determine the horizontal distance through which infection may spread, I found that susceptible plants, grown in a flat with several infected by wart, remained free from infection for two months if separated by an interval of 6 inches, although in watering the soil surface frequently was flooded. It appears that spore-liberation must occur immediately adjacent to a susceptible

<sup>2</sup> In a paper received after this manuscript was completed (Köhler, E. Beiträge zur Keimungsphysiologie der Dauersporangien des Kartoffelkrebserregers. Arb. Biol. Reichsanst. Land.-Forstwirtschaft. 13: 369-381. 1924) data are presented to show that freezing is without effect in abbreviating the rest period, and that tap water is as effective a medium for germination as a compost extract or as various dilute acids.

host part, and penetration must begin within about an hour for infection ordinarily to result.

This assumes that zoöspores which do not soon gain entrance into a susceptible host perish. A possible alternative is their passage into an amoeboid stage which may defer this fate by a temporary saprophytic existence, or the zoöspores might encyst, as myxomycete swarm-spores readily do. An amoeboid condition as a sequel to active swarming has been described by certain investigators and questioned by others, but the occurrence of such a stage as a characteristic feature of the life history awaits proof. Having regard for the high type of parasitism exhibited by *S. endobioticum*, the intervention of a saprophytic stage seems unlikely. Brierley (6) states that attempts at Rothamsted to cultivate this fungus *in vitro* "have not given hope of immediate achievement."

The long persistence of the disease in infested soil may be accounted for without hypothecating a saprophytic stage or a series of germinations and encystments. One of the longest authentic records of persistence is that by Schaffnit (42), who found that wart still occurred when potatoes were planted in an infested plot which for nine years had been in sod.

In a study supplementary to the present investigation, Dunegan<sup>3</sup> found that resting sporangia of *S. endobioticum* could be isolated from the soil of infested gardens 8 to 9 inches below the surface, and in planting tests soil taken from the 6- to 7-inch level resulted in infection. Goss (12) has found that buried seeds of many common weeds may persist ungerminated but viable for 20 years.

All the field and experimental evidence indicates the importance of oxygen in the germination of the resting sporangia. When the effect of depth, packing, and moisture content on the oxygen-supplying power of the soil (16) is taken into account, particularly when these tend to be cumulative as in an undisturbed sod, it seems reasonable to attribute the longevity of buried seeds and spores to low accessibility of oxygen. Since resting spores of *S. endobioticum* may retain their vitality for over four years when artificially stored (8), it is possible that they may remain viable in the soil for at least as long a period.

### Entrance of the Parasite and Effect on the Host

*Synchytrium endobioticum* gains entrance into its hosts by direct penetration of epidermal cells by the motile spores, as described in detail by Curtis. In the process of penetration no cytolytic effects were observed, and apparently only mechanical forces are involved. The immediate response of the invaded cell is simple hypertrophy if the infecting body is a zoöspore, and hypertrophy accompanied by cell division if this body is a zygote. In both cases the stimulus is exerted more or less on the adjacent cells also, though the hypertrophy induced by a single prosorus involves

<sup>3</sup> J. C. Dunegan, unpublished data.

only a small group of epidermal cells (figs. *B*, *E*, Pl. XLII; fig. *E*, Pl. XLIII). In the first case the development of the parasite is entirely peripheral so that liberation of the zoöspores to the exterior is unimpeded; the hyperplasia induced by the zygote carries the parasite to a depth of several cell layers and at the same time increases the surface of meristematic tissue, which is favorable to reinfection. This nice adaptation of host response and infection has been commented on by Potter (31) and Artschwager (3). Therein is to be sought the explanation of the extremely rapid development and large size of the overgrowths as compared with other plant galls.

## THE ENVIRONMENTAL FACTORS OF INFECTION

### Previous Observations

No experimental study of the relation of external factors to wart infection has been reported. Observations on the distribution of the disease and its severity under different weather conditions have been somewhat contradictory, but agree in the main that infection is favored by abundant rainfall and retarded by high temperature. The disease attains its highest incidence in the British Isles and northwestern Germany, and in these general areas is, as a rule, more severe as the climate becomes cooler and wetter. Wollenweber (57) makes note of the fact that in Europe wart is found only above the 50th parallel, extending from the British Isles to Poland and from Norway to Hungary. In America it extends as far south as the 35th parallel, but in a region of high altitude. It is evident that climate has not alone determined its distribution, since parts of Germany which are infested are relatively warm and dry, whereas Denmark and Sweden, with cooler and moister climates, and lying apparently in the path of spread, are relatively free from the disease. Similarly France appears to have escaped, although its channel coast differs climatically in no essential way from southern England and Holland (53). Even in England, where the relative freedom of the South and East was for long attributed to the scantier rainfall there than prevailed in the West and Midland, Taylor (49) attributes greater influence to the source and methods of distribution of seed potatoes.

The wart survey in the United States has shown no correlation between soil type and incidence of the disease,<sup>4</sup> and experimentally it has been produced in a variety of soils, including peat, sand, loam, and clay.

### Climatic and Soil Factors in the Freeland Area

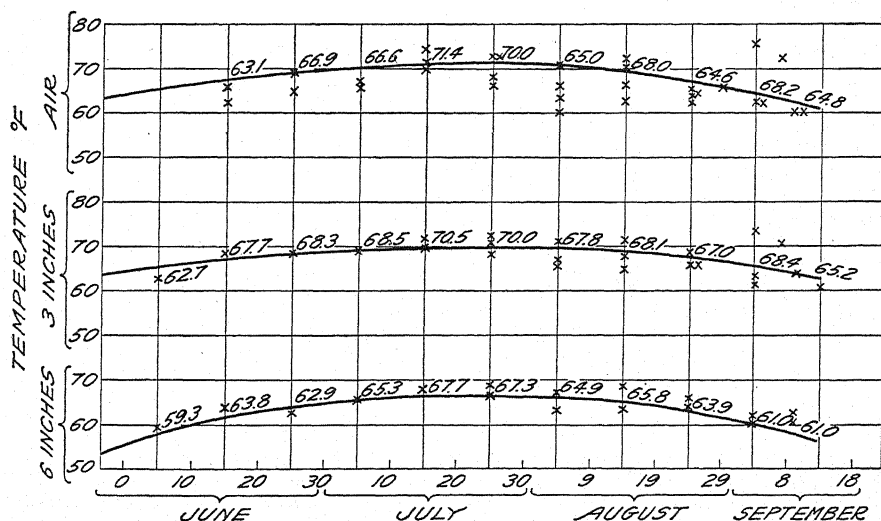
The infested areas in the United States are distributed through eastern and central Pennsylvania, western Maryland, and northeastern West Virginia, following approximately the distribution of the anthracite and

<sup>4</sup>Lyman, G. R. Report on the potato wart survey of 1921 conducted by the Plant Disease Survey. MS.

bituminous coal fields (22, 54). More striking than any correlation of distribution with climate or altitude is the occurrence of wart mostly in non-agricultural areas, where potato-growing is carried on in back-yard gardens for strictly domestic consumption. Here potatoes have been an annual crop for years, and the wastage from one crop has been turned back into the soil for the next, affording the maximum opportunity for persistence of a disease like wart. This, together with the former practice of using inferior table stock for seed, much of which came in from Europe before the foreign potato quarantine took effect, suffices to account for the establishment of the disease in only this limited area.

The gardens in which wart occurs exhibit great diversity of soil type. Derived originally from the weathering of a red shale, slightly acid in reaction, the soil has been modified by the introduction of ashes, stable manure, and vegetable *débris*. The soil is moderately retentive but well drained, losing its supracapillary water quickly after each rain. The reaction of the soil solution varies from slightly below pH 4 to pH 8.5, most of the readings centering at 6 to 6.5. The weed flora is predominantly of calcifuge types.

There is a coöperative station of the U. S. Weather Bureau at Freeland, Pennsylvania, and one was formerly maintained at Drifton, 2 miles distant. The combined records of these two stations extend over 25 years. The monthly and seasonal mean temperature, precipitation, and number of days with rain are shown in table I.



TEXT FIG. 1. Seasonal march of air temperature 1 foot above, and soil temperature 3 and 6 inches below the soil surface in a potato plot at Freeland, Pa. The curve is based on the mean temperature for 10-day periods computed from continuous thermograph records in 1919 to 1922.

Thermograph records in representative potato plants have been kept since 1919. The mean values for 10-day periods have been calculated from the continuous records and are shown in text figure 1 for the 6- and 3-inch levels below the soil surface and for 1 foot above. From these means a free-hand smoothed curve has been drawn to show the seasonal march of temperature. The mean daily range of air temperature and that at the soil surface is about 15° F., and the extremes during the growing season are from 42° to 93°. Maxima above 90° may occur at any time from June to September.

TABLE 1. *Meteorological Conditions at Freeland and Drifton, Pa.*

	May	June	July	August	September	Growing Season
<i>Freeland</i>						
Mean temp., °F. (1920-24).....	54.7	65.9	68.7	65.1	59.8	62.4
Rainfall, inches (1915-1924).....	3.96	5.10	5.47	4.37	4.31	23.23
No. days with rain.....	12	10	10	11	13	56
<i>Drifton</i> , 20-year average						
Mean temp., °F.....	56.6	64.2	68.5	66.3	59.3	63.0
Rainfall, inches.....	4.51	4.18	4.51	5.11	3.60	21.91

### Field Observations on the Relation of Meteorological Factors to Infection

*At Freeland.* In 1920 a field experiment was conducted to ascertain the effect of varying amounts of soil moisture on infection. A portion of one row of potatoes was subirrigated with a constant slow flow of water. A second row was provided with a canvas cover arranged on rollers to slide into place at the beginning of each rain, being released by a filter-paper trigger which tore when wet. The device was fairly successful in limiting rainfall on the protected row to the small amount that fell before the trigger was released, except on one occasion when the soil was thoroughly wet in the surface inch or two.

TABLE 2. *Effect of Irrigating and Protecting from Rain on Wart Infection in Naturally Infested Soil*

	Soil-moisture Content		Yield of Tubers (lbs.)		
	Range (percent)	Mean (percent)	No. Plants	Healthy	Infected
Irrigated row.....	16-58	35	20	8	14
Covered portion of row.....	10-20	12	8	7	3/4
Exposed portion of row.....	15-50	32	10	3	7

The wetter soil did not result in more infections, but a single thorough wetting sufficed to cause infection in the dry row.

In April, 1921, partly grown plants were set in infested soil at Freeland, so that by June tuber-formation had begun—a month earlier than normal.

June proved to be excessively warm, following a dry May, and drought conditions prevailed throughout the month except for heavy rains on the 4th and 12th. Observations on the initiation of infection in relation to rainfall showed that the moisture supplied on these two days caused the germination of enough spores which had rested over winter to infect every susceptible plant then present. Following infection, development of the overgrowths proceeded normally though the soil temperature ranged between 68° and 78° F.

*At Washington, D. C.* An experimental planting to determine the virulence of the wart fungus under weather conditions at the southern limit of main crop potato-production was made in an outdoor inclosure on the grounds of the Department of Agriculture in 1923. Lightly infected tubers were set in wart-free soil, and clean tubers were planted in soil to which dry wart was added. The mean temperature and precipitation in May were 63.4° F. and 1.5 in.; in June, 75.6° and 2.8 in. Five plants out of 72 were infected by July 5.

The experiment was repeated in 1924. Forty-five plants of 3 susceptible varieties were set on April 21. May was exceptionally cool and wet, but no infection was visible up to June 1. On June 13 warty growths appeared above ground, and on June 24 every plant was found severely infected. Successive plantings were made throughout the summer, but no new infections were noted between June 24 and August 17. A final examination was made October 10, when 14 remaining plants were all infected.

Had the plantings been carried out according to the usual practice in this locality of an early and a late crop which occupy the ground, except for a brief period in midsummer, from April to November, both crops would have been infected. The principal meteorological features of this season are shown in table 3.

TABLE 3. *Temperature and Precipitation at Washington, D. C., May to September, 1924 \**

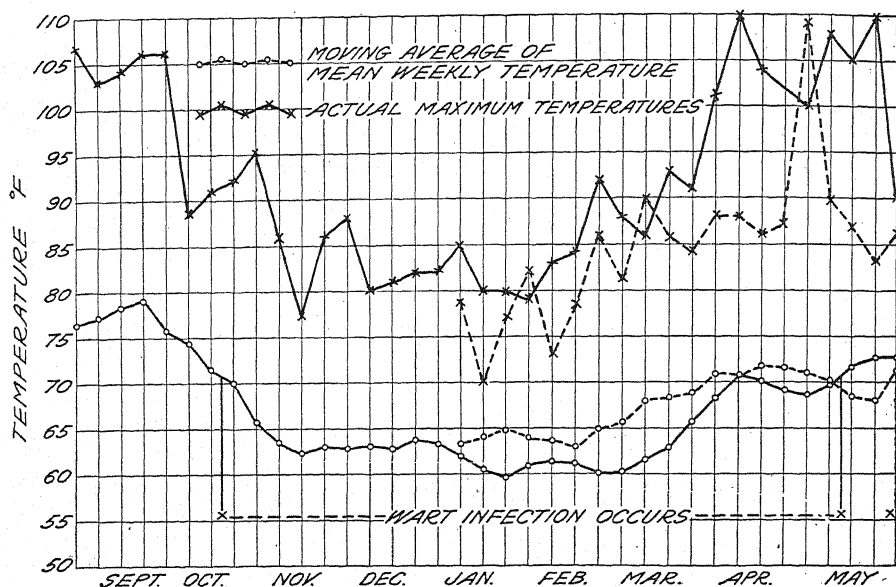
Month	Temperature, Degrees F.			Mean Soil Temperature, 1 Inch †	Precipitation, Inches
	Mean	Mean Maximum	Mean Minimum		
May.....	60	69	51	—	6.73
June.....	71	81	62	78	3.89
July.....	75	85	66	83	2.76
August.....	75	85	65	80	5.07
September.....	64	73	55	67	7.86

\* Air temperature and precipitation data from U. S. Weather Bureau.

† Thermograph record beginning June 24.

In the greenhouse a marked decline in the success of inoculations occurs with the approach of summer. This is attributed to the rapidly rising temperature beginning early in April, resulting in diurnal temperatures of 80° to 90° F. on all bright days. Test plantings were made at fortnightly

intervals to determine when natural infection ceased. From three years' observations it appears that potatoes planted later than April 1 do not make sufficiently normal growth to be readily infected, and infection of earlier-set plants ceases between the middle and the last of May. In text figure 2 is shown the seasonal march of temperature in the spring and fall of 1922 and the spring of 1923, as determined by computing the moving average of the weekly mean temperatures derived from continuous thermograph records. The position of the weeks in the fall and spring when the first and last natural infections were noted is indicated.



TEXT FIG. 2. Seasonal march of temperature in a greenhouse, showing the approximate duration from October to May of temperature suitable for natural infection by potato wart. Curve for 1922-23 solid line, for 1924 broken line.

### Experimental Infection: Methods of Inoculation

In the present investigation attempts have been made to infect growing plants and sprouting tubers by inoculating with germinating sporangia of both kinds. Great variability in the behavior of different lots of resting sporangia has been encountered, and material taken in sequence from the same lot at different times has given variable results. As a source of resting spores, partially disintegrated wart tissue collected from the field was preserved without allowing it to become air-dry, or fresh warts were dried slowly indoors, avoiding both extremes of wet decomposition and rapid desiccation, which impaired viability. In material thus dried probably many of the sporangia are interrupted in the process of maturing, and prolonged soaking is necessary to induce germination. On the other hand,



germinating soral sporangia are readily obtained by immersing thin peripheral slices of tumors in water, or by pinning them to susceptible parts such as tuber eyes and stolon buds.

Tubers were prepared for inoculation by sprouting in steamed sphagnum at about 22° C., sparingly watered. Young sprouts are essential, and are in best condition for inoculation when about 2 mm. long. Growth may be allowed to proceed to root- and stolon-formation, after inoculation of which the seed piece is transferred to soil or to a solution culture. Pot-grown plants also were used to obtain leaves, shoots, and young tubers for inoculation, and to determine the effect of different stages in development on susceptibility to infection. The subterranean parts were exposed by washing out part of the soil, then after inoculation recovering with moist cotton and sphagnum. An unsuccessful attempt was made to study infection of tubers under constant conditions of temperature and humidity by sprouting in shallow dishes of sphagnum, or wrapping in Canton flannel, then placing them in seed germinators. As inoculum, germinating sporangia of both kinds were used, also fragments of dry and fresh warts were attached to the eyes, and the preparations syringed daily. The use of alternating temperatures also was tried. Several hundred tubers were inoculated in this way, but infection occurred very infrequently and only within the temperature range of 18°–22° C. On the other hand, infection of sprouting tubers under sphagnum was successful when the dishes were exposed to the free circulation and variable temperature of the greenhouse and laboratory. Whether the effect was primarily on the potato or was divided between host and parasite is unknown, but most of the tubers sprouted in the seed germinators showed evidence of respiratory difficulty by lenticel proliferation, which was extreme above 23° C.

In all the inoculation experiments there was a larger proportion of failure than one expects when working with fungi which sporulate abundantly and whose spores germinate by germ tubes. This was attributed to the delicacy and slight mobility of the infecting body of *Synchytrium*.

### Germination of Sporangia

Resting sporangia were isolated by the sieve method described by Curtis, also by rubbing dry wart material, free from soil, through a 200-mesh screen; and with a small pipette under a binocular microscope. They were mounted in hanging drops, and an attempt was made to determine the thermal range for germination by placing them in incubators at different constant temperatures. This was found impracticable owing to the length of time required for breaking the rest period, being a matter of a number of days or weeks. Actual exit of the zoöspores is of short duration (Curtis found liberation complete in 10 to 20 minutes), but the number of empty sporangia appearing after an interval furnishes a workable criterion of germination. In this work, however, mass cultures of sporangia were

employed, and infection, following their transfer to susceptible plants, was made the criterion.

Curtis found the activity of the soral zoöspores retarded by temperature above 20° C., and observed that, whereas the processes antecedent to germination continue when the sporangia are kept only moist, free water is required for rupture of the sporangial wall and exit of the zoöspores. Similarly in the present study, emptied sporangia were observed only in actual water drops, never when they were surrounded merely by a thin film.

Resting sporangia were barely immersed in shallow dishes which were set in incubators affording a temperature range of from 10° to 30° C. After periods of soaking varying from two hours to several days, a few drops of the clear liquid were removed with a pipette and placed on susceptible potato parts, usually stolon buds on growing plants. Infection occurred only after soaking for at least 2 to 3 days, though germination may continue during longer periods, or spores may fail to germinate but remain viable. This is shown in table 4.

TABLE 4. *Effect of Prolonged Soaking of Resting Sporangia at Various Temperatures on Subsequent Infection*

Temperature, ° C.	Time of Soaking, Days.	Infection Noted in Days
20-25.....	7	15
10.....	9	30, severe
15.....	9	30, severe
20.....	9	30, severe
25.....	9	30, moderate
30.....	9	60, slight
14 ± 2.....	11	40, severe
25 ± 2.....	11	40, moderate

In table 5 the results of several attempts to inoculate growing potatoes with germinating resting sporangia are given. The negative results were not distributed in such a way as to indicate either the complete or the optimum thermal range, and the table therefore shows only some of the conditions under which infection may occur.

TABLE 5. *Effect of Temperature and Period of Soaking on Infection by Germinating Resting Sporangia*

Treatment		Incubation	Infection	
Temperature, ° C.	Period, Hours	Temperature, ° C.	Plants	Days
10	48	16	1	2
18-20	48	16	5	2-9
18-20	48	22	1	4
18-20	48	27	1	2
18-20	60	27	3	3-6
22-25	24	16	2	2-5
28-30	48	16	1	2
28-30	48	27	1	2

The earliest stages of infection were not always observed, but they were usually visible with a hand lens in 2 to 4 days. Following infection, some of the plants were kept under observation for several days and the overgrowths were measured at intervals with a Vernier caliper. In table 6 a series of such measurements is given to show the rate of increase in size of two tumors, and in figures *A* to *C*, Plate XLIV, three stages in their growth are illustrated.

In their youngest stages the tumors are nearly spherical. Because of the difficulty of obtaining measurements on three perpendicular axes without injuring the tissue, two measurements approximating the greatest and least diameter were obtained and the mean of these two was arbitrarily taken as the third diameter in computing volumes.

TABLE 6. *Rate of Growth of 2 Tumors; Inoculation 4 P.M., February 13, 1922; Infection Visible 9 A.M., February 16*

Time after Infection	Size (mm.)		Approximate Ratio of Volumes in 1
	1 *	2	
68 hrs.....	about 2 x 2	about 1 x 1	8
98 hrs.....	3.4 x 3.1	2.6 x 2.0	34
127 hrs.....	4.9 x 4.1	2.9 x 2.2	91
149 hrs.....	5.1 x 5.0	3.2 x 3.0	129
172 hrs.....	6.2 x 5.1	3.8 x 3.5	180
9 days.....	7.4 x 7.0	5.4 x 5.2	373
12 days.....	16.4 x 8.2	10.5 x 9.0	1,860
19 days.....	26.8 x 22.4	18.5 x 17.0	14,800

\* The figures refer to the tumors numbered 1 and 2 in figures *A* to *C*, Plate XLIV.

During the first two days following discovery of tumor 1, the ratio of volume increase for each 24 hours was over 3, and for the next two days it was about 1.5. In 16 days the volume increased over 1800 times. In the normal tuber, Clark's (7) data show that the ratio of average weight increments per week, beginning with tubers weighing about 12 g., descends in an order something like 3, 2, 1.6, 1.3, etc. The greatest increase in weight per tuber during a three-week period is about eight times. Clark's data apply to a later period in the development of the tuber, when the rate of increase in weight and size is much less than at the beginning of the tuberization; and, because of the spongy texture of the overgrowths, the same correlation between weight and volume would not hold in normal and tumor tissue. Nevertheless, the great stimulus to growth rate resulting from wart infection is evident.

#### Thermal Range of Infection for Germinating Resting Sporangia

In table 7 are assembled the principal results of inoculation experiments with germinating soral sporangia. Similar results were obtained by inoculating various parts of growing plants; thus, leaves were infected by

immersing the tip of a branch in a shallow dish containing germinating sporangia, and axillary shoots and stem internodes were infected by pinning a fragment of fresh wart in the axil, then keeping it wet by frequent syringing.

TABLE 7. *Effect of Temperature on Infection of Sprouting Tubers by Germinating Soral Sporangia*

Temperature during Inoculation, ° C.	Period of Exposure (hrs.)	No. Infected in Relation to No. Grown	Remarks
0.6 ± 0.2	6½	1/2	1 rotted
4 ± 2	18	2/2	
4 ± 2	24	1/2	
10	3	0/2	
10	5½	3/3	
10	24	2/2	
12	3½	0/2	
14 ± 2	48	2/2	
15	25	2/2	
17 ± 2	5	1/2	
20	6½	2/2	1 rotted
20	24	1/2	
21 ± 1	24	1/2	1 rotted
22 ± 1	3	2/2	2 rotted
24	24	0/2	
25 ± 1	48	1/2	2 rotted
27 ± 2	24	3/6	
28 ± 1	5	0/2	1 rotted
28 ± 1	6½	1/2	
30 ± 1	3	2/2	
30	5½	0/3	
30	24	0/2	

Sprouting tubers and the buds of stolons or the base of the stem were found to be the most susceptible parts. The eyes are susceptible at an early stage of tuber-development but seem to acquire resistance with age. When more or less full-grown tubers were inoculated in the eyes, only weak infections developed, or they resisted repeated attempts to infect them. In the field such infections are frequent, probably because of the more rapid development of tubers as compared with greenhouse-grown plants. It is a general relation that the greater its meristematic activity the more susceptible is a given part; but aside from roots any tissue may, at times, be susceptible. Weakly growing plants are not, however, always resistant, since in some attempts at chemical soil-disinfection considerable injury to the plants occurred without preventing infection.

To obtain an idea of the approximate length of time during which liberation of zoospores from soral sporangia may continue, small fragments of fresh tumors were pinned to the eyes of sprouting tubers and immersed in water at 11° for two hours. They were then transferred to a second set of tubers for a like period. This was continued at intervals for 32 hours. As soon as the inoculum was removed from the tubers, they were rinsed in

water and placed in moist sphagnum. In table 8 is shown the effect of this treatment on infection.

TABLE 8. *Period during which Liberation of Zoöspores from Soral Sporangia is Active, and Relation of Length of Exposure of Tubers to Severity of Infection*

Treatment of Inoculum		Time of Exposure of Tubers (hrs.)	Infection
Temperature, ° C.	Period of Soaking (hrs.)		
I2	2½	2½	Moderate
II	5	2½	Moderate
II	7½	2½	Severe
II	9½	2	Moderate
II	23	17	Severe
II	25	2	Moderate
II	32	2	Moderate

Although the experiments described in the foregoing section were not carried out on a scale adequate for the complete determination of the thermal range for germination and infection, they show that, with the experimental methods employed, infection may occur from near the temperature of melting ice to 30° C. and that the optimum range is from 15° to 20° C. This transcends the temperature limits within which natural infection occurs and also those favorable for growth of the host.

### Effect of Soil Temperature on Infection

Before it was considered advisable to remove infested soil to Washington, D. C., for experimental work, a number of experiments were carried on at Pennsylvania State College, in coöperation with Prof. C. R. Orton. Several types of apparatus for controlling soil temperature, including Wisconsin tanks (18), were tried, and the approximate effect on infection of a temperature range of 18° to 33° C. was determined. Infection was noted at a mean temperature as high as 27° C., but only when fluctuations down to 21° or lower occurred.

In tables 9 and 10 the results of experiments carried out in Wisconsin tanks at Washington are given. In these experiments the soil cans were adjusted to a uniform tare weight, then filled with the same quantity of soil previously brought to the desired moisture content. Seed pieces of uniform weight and with sprouts 4 to 5 inches long were set one in each can. The soil surface was 1 inch below the water level in the tank and was covered with an inch of asbestos fiber or granulated cork. The moisture content was restored to the original amount by watering three to six times a week, water of the same temperature as the soil being poured over the soil surface. For reasons given later, this method of watering was considered preferable to introducing the water below the seed piece. Continuous thermograph records were kept, or the temperature of the water was noted thrice daily; from these records the mean temperature and the extreme range were computed.

TABLE 9. *Effect of Soil Temperature on Infection Oct. 6 to Nov. 17, 1923. Soil-moisture Content 50 to 75 Percent of Saturation. Air Temperature about 20° C. Two Plants at 30° and 5 at 33° Died Prematurely*

Mean Temperature, ° C.	Extreme Range, ° C.	No. Plants	No. Infected	Tuber-formation
10.1	5-14	8	0	Normal
15.1	13-17	8	2	Normal
18.4	15-22	8	3	Normal
25.8	23-30	8	0	Mostly thickened stolons
29.1	25-34	8	0	Mostly thickened stolons
32.2	28-35	8	0	None

TABLE 10. *Effect of Soil Temperature on Infection Dec. 15, 1923, to Jan. 21, 1924. Moisture Content 46 to 75 Percent of Saturation. Air Temperature, 19.6° C.; Range, 12°-34°. Three Plants at 20° Dug Prematurely*

Mean Temperature, ° C.	Extreme Range, ° C.	No. Plants	No. Infected	Tuber-formation
13.0	11-15	6	6	Normal
15.6	15-16.5	6	6	Normal
19.7	18.5-21.5	6	2	Normal
23.4	20-26	6	0	Thickened stolons
26.6	23-31	6	0	Thickened stolons
30.2	27-34	6	0	Thickened stolons

Several similar experiments using larger containers were carried out. Two-gallon crocks were placed in a shallow water bath, the soil level being about 2 inches below the water, and covered with a 1-inch layer of asbestos or cork. The first experiment was run from April 26 to June 4, 1923, 10 crocks being maintained at about 25° C. (range 18.5° to 30.7° C.). The soil-moisture content was 80 percent of saturation. In half the pots in each set water was poured directly on the surface, and in half it was poured through a tube to a porous reservoir in the bottom of the crock, thence distributed by capillarity. The plants were killed by high-air temperature before completing their growth. Small tubers set but no wart developed.

TABLE 11. *Same as Table 10*

	50% Saturation		75% Saturation		Watered Freely
	24° C. Con- stant Temp.	Variable Temp.	24° C. Con- stant Temp.	Variable Temp.	Variable Temp.
No. plants.....	9	1	9	1	2
No. plants infected...	0	0	1	0	2
Tuber-production.....	Subnormal	None	Normal	Normal	Normal

A second attempt with the soil temperature at approximately 25° and 30°, and the moisture content 50 or 80 percent of saturation at each temperature, resulted similarly. In a third, the temperature of the water bath

was  $24 \pm 3^{\circ}$  C., and half the crocks were 50 percent and half 75 percent saturated. One crock at each moisture content was exposed to the variable greenhouse temperature, and a porous pot similarly placed but freely watered served as a further control.

From the various experiments on the relation of nearly constant soil temperature to wart infection it appears that the upper limit is near  $24^{\circ}$  C., infection occurring then only if the soil moisture is relatively high, that is, 60 to 75 percent of saturation. The lower limit probably is not much below  $12^{\circ}$ . The optimum is about  $15^{\circ}$ . When the thermal range for infection is compared with that for spore-germination and for growth of the host, it is found to coincide more nearly with the latter than with the former. Richards (32) found that the optimum temperature for development of the aerial parts of the potato plant is somewhat above that for tuber-production, being about  $21^{\circ}$ – $23^{\circ}$  for sprouting and growth of sprouts, and about  $18^{\circ}$  for yield of tubers, taking the entire growth period into consideration. Jones, McKinney, and Fellows (19) state that the optimum soil temperature for production of green weight and number of stems per hill is near  $21^{\circ}$ , and that the largest tubers are produced between  $15^{\circ}$  and  $22^{\circ}$ , with the optimum at or near  $18^{\circ}$ .

#### Relation of Soil-moisture Content to Infection

No satisfactory method of controlling this factor was found if one considers not only quantity of soil water but its distribution as well. The Livingston cone-shaped auto-irrigator was first tried, and three experiments were completed. In these, 1-gallon crocks were used as containers, each supplied with one auto-irrigator. A U-shaped bend in the supply tubes containing mercury valves of different lengths served to regulate the intake of water. By this means a total moisture content of 15, 20, 30, 40, or 50 percent was approximately maintained in a soil having a maximum water capacity of about 50 percent. The crocks were covered with wax seals. The distribution of water was very uneven. The potato plant is shallow-rooted, particularly in pots. This characteristic reduced the moisture content of the upper level of soil faster than it was replenished by capillary transfer from below, a condition only slightly improved by placing the irrigator cone even with the soil surface. On the other hand, when the seed piece was placed near the bottom of the crock it invariably rotted if the soil moisture was high.

Tuber-production was not retarded because of the wax seal, the plants producing about as well as similar plants in porous pots of the same size. However, in all crocks with high moisture content the tubers showed enlargement of lenticels indicative of oxygen deficiency, a behavior correlated with an unfavorable predisposition for infection.

In only two crocks watered by auto-irrigators did infection occur. These did not have wax seals, and it is possible that they were at some time

wet by drippage. The average moisture content in these crocks was 34 percent, or 77 percent of saturation, but the soil was wetter towards the bottom of the crock and dryer towards the top.

Another series of experiments was carried out, using 2-gallon crocks watered from a reservoir consisting of a 3-inch porous pot placed on the bottom and sealed to a glass tube projecting above the soil. The surface was sealed with a layer of wax. These crocks were maintained by frequent weighing and replenishment of transpired water at 30, 40, 60, and 70 percent of saturation. Growth of potatoes was better than in the smaller crocks, but, though normal stolons and tubers were produced, wart infection failed.

Finally three containers of about 150 kg. capacity were filled with soil previously made up to approximately 30, 60, and 90 percent of saturation, thus containing sufficient water at the outset for the entire growth period. The cans were closed by metal covers perforated for four sprouts. Lightly wart-infected seed was planted, but the infections dried up without spreading.

These negative results afford considerable evidence that one condition for infection is the presence of free water in the soil, but that a state approaching constant saturation is unfavorable to the host and *pari passu* to the parasite. Alternate wetting and aëration seem to be most favorable for infection.

A type of metal container of about 15 kg. soil capacity, provided with an annular reservoir opening just below the soil surface, also was tried. This overcame the objection of watering entirely from below and permitted more even distribution of water than when water is poured over the surface. Water was added to the reservoir weekly. The results of one experiment are given in table 13.

TABLE 12. *Relation of Soil-moisture Content to Wart Infection. Each Set Contained 4 Cans, Each with 4 Plants*

	I	2	3
Initial moisture content . . . . .	23 %	25 %	32.5%
Mean deviation from initial content in 5 weeks . . . . .	1.9 %	2.9 %	2.9 %
Final moisture content in upper 3 in. of soil . . . . .	10.3 %	10 %	12.1 %
No. of plants infected in relation to no. grown . . . . .	0/12	0/10	2/12
Yield of tubers, average per plant . . . . .	27 g.	27 g.	24 g.

Infection was limited to the set having the highest soil-moisture content and also requiring the greatest addition of water at each watering. This set was slightly below the others in yield of tubers.

The results of another experiment also illustrating the dependence of infection on the periodical presence of free water in the surface layer of soil are given in table 13. To avoid the difficulty of obtaining normal potato plants in soil constantly near saturation, the plants were started in two sets of crocks at about 40 and 60 percent saturation, respectively, and



all were watered from below. At the end of ten weeks, the moisture content was increased and the water supplied either from below or applied to the surface as shown in the table.

TABLE 13. *Relation of Soil-moisture Content and Manner of Watering to Wart Infection. Maximum Water Capacity of the Soil, 48%*

Set No.	Moisture Content during 1st 10 Weeks, % Saturation	Moisture Content during 2d 10 Weeks and Method of Watering	No. Plants Infected in Relation to No. Grown	Tuber-production
1 a. ....	39	39; above	0/2	30 g.
b. ....	39	39; below	0/2	78 g.
c. ....	39	50; above	0/2	65 g.
d. ....	39	50; below	0/2	17 g.
e. ....	39	60; below	0/2	42 g.
				Total, 232 g.
2 a. ....	57	57; above	0/2	28 g.
b. ....	57	57; below	0/2	30 g.
c. ....	57	80; above	1/2	64 g.
d. ....	57	80; below	0/2	37 g.
e. ....	57	90; below	0/2	21 g.
				Total, 180 g.

Only one plant was infected in soil maintained at about 80 percent of saturation by watering from above. The total yield of tubers in all pots with soil more than half saturated is significantly lower than that in the drier series.

The results show infection to be dependent on high soil-moisture content or on periodic flooding of the soil. To differentiate the effect of these two factors, the following experiment was planned. Ninety-six potato plants in 4-inch pots were divided into four lots and each was plunged to the rim in sand in large metal trays. One set was brought to a moisture content of 13.5 percent, two of the others to about 20 percent, and the fourth to about 30 percent. In percentage of saturation the moisture contents were respectively 27, 40, 40, and 60. The plan was to water all pots of the first two sets from the surface, also maintaining the sand at the respective moisture contents, whereas all the water of the other two sets was applied to the sand only, whence it passed through the pots to the plants. Water was added daily, and twice a week soil samples were taken from certain pots for a moisture determination.

It is evident that the manner of watering exerted much more influence on infection than the soil-moisture content; thus, in sets 2 and 3, with nearly the same moisture content, the amount of infection is about 90 percent and 0, depending on whether the surface is flooded in watering or not. In set 4, with more than double the moisture content of set 1, the number of infected plants is about the same.

Previous experiments on the relation of soil moisture to infection showed a higher yield of tubers in the drier pots and marked depression

TABLE 14. *Relation of Soil-moisture Content and Method of Watering to Wart Infection*

Set No.	1	2	3	4
Manner of watering.....	Directly	Directly	Through sand	Through sand
Initial moisture content.....	13.5%	22 %	21 %	32 %
Mean moisture content for 14 weeks...	13.6%	20.5%	19.7%	28.4%
Percent saturation.....	27 %	42 %	40 %	58 %
No. plants infected in relation to no. grown.....	8/24	21/24	0/24	7/24
No. separate infections.....	17	106	0	14
Weight of infected tubers.....	16 g.	235 g.	559 g.	401 g.
Weight of healthy tubers.....	12 g.	138 g.	—	67 g.
Total.....	28 g.	473 g.	559 g.	468 g.

as the soil approached saturation. In this experiment the yield in the moister sets greatly exceeded that in the driest, but the highest soil-moisture contents were of the same order as the lowest previously. Wart infection may occur in soil that is on the average too dry to support a productive potato crop, provided water is supplied so that the soil surface is periodically wet.

#### Effect of Soil Reaction on Infection

Among the first control measures suggested for wart was the addition of quicklime to infested soil (24). Others observed that liming had no effect; indeed, Malthouse (23) found the disease more severe where old plaster had been dumped. Schaffnit (44) obtained no appreciable control from the application of 1320 pounds of sulfur per acre. Potter (31) believed that infection could be suppressed by a degree of alkalinity not inimical to potatoes; thus, infection failed when the reaction of the soil was changed by liming or by addition of soda from pH 7.5 to 10.5, although the yield also was reduced. No infection was observed in any pot giving a reaction of over pH 8.5 at harvest.

In tests of a number of chemical soil treatments at Freeland, no relation between the reaction produced and the effectiveness of the chemical against wart was noted (15). However, sulfur at the rate of  $2\frac{1}{2}$  tons or more per acre, sodium carbonate at the rate of 40 tons, and sulfuric acid-bichromate cleaning mixture, 1 or 2 gallons per square foot, caused inhibition of wart for three years, but in none of these treatments was the soil reaction precisely determined.

Preliminary experiments to determine the course of infection in a series of soils artificially adjusted to various reactions were begun in 1921. Lime, sodium carbonate, various acid phosphates, acid sulfates, and sulfur were tried, but did not afford a sufficiently wide range of reaction. However, no wart developed in pots receiving heavy applications of lime, sodium carbonate, or sulfur, though the reaction, as determined colorimetrically, did not transcend the range pH 3.8 to 7.3.

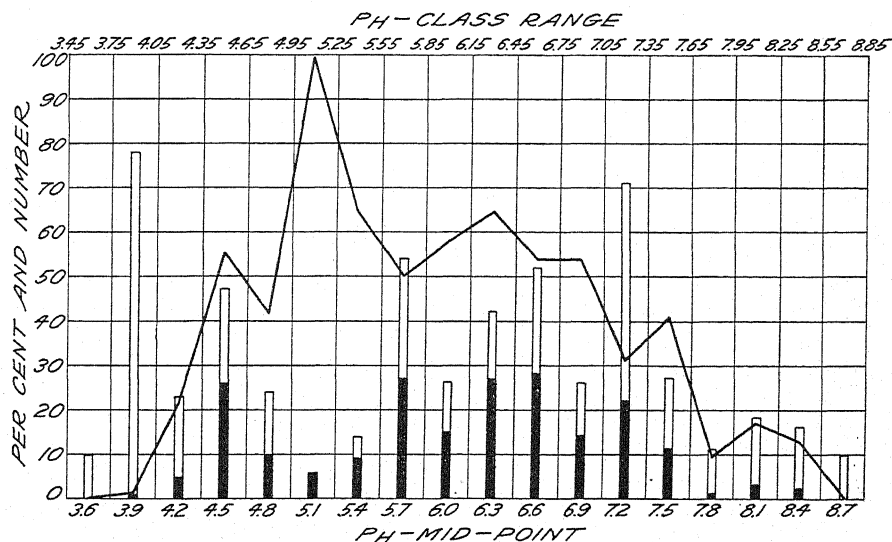
Next a more extensive series of treatments was begun, and the electro-

metric method of determining H-ion concentration was adopted to eliminate difficulties with turbid solutions. Consecutive plantings were made through three years, or until infection consistently occurred in each treatment.

Each treatment was replicated three to five times in new 8-inch clay pots. The fertilizer mixtures, sets 36 to 41, had the following composition in grams per kg. of soil:

Number	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	CaH PO <sub>4</sub>	KHSO <sub>4</sub>	N-P-K ratio
1.....	.18	.26	.26	5-8-10
2.....	.21	.31	.18	5-8-6
3.....	.25	.37	.07	5-8-2

In determining H-ion concentrations, a composite sample of 10 g. was taken with a soil-sampling tube from three points in each pot, shaken up with 50 cc. of distilled water and allowed to stand one hour, then read without filtering. Duplicate determinations usually agreed closely, as did the different pots of each set, the variability seldom exceeding 0.2 pH.



TEXT FIG. 3. Relation of soil reaction to wart infection. The columns show the number of plants grown in each pH class, and the shaded portion the number infected. The curve shows the percentage of infection.

From four to five separate determinations were made on each set which was carried through two years. The results are shown in table 15 and text figure 3. The sub-letter *a* in certain sets indicates that the soil had previously received the same treatment as the set bearing that number.

The highest H-ion concentration at which infection was noticed was

pH 3.9, the lowest, pH 8.3. These can not be interpreted as precisely the limits of tolerance for H or OH ions, since inhibition may be the resultant effect of the ions taken together; furthermore, the soil in some naturally infested gardens was found to vary from pH 4 to pH 8.5. However, this probably approaches the extreme tolerance of the fungus for the H ion, and growth of the host occurs in only a slightly wider range, chiefly on the alkaline side. The curve in text figure 3, of the percentage infection at different soil reactions, suggests an optimum near pH 5.0.

An attempt was made to determine the effect of reaction of the medium on germination, by soaking resting sporangia in solutions adjusted with buffer tablets to a range of pH 3 to pH 8, also in .001 N HCl and other acids, and in .001 N NaOH. The presence of only a small quantity of wart tissue tended to change the reaction of even the buffered solutions in the direction of neutrality. However, spores which had been soaked for three days in .001 N HCl or in any of the buffered solutions subsequently were able to cause infection, but the alkali was fatal to them.

TABLE 15. *Effect of Soil Reaction on Infection*

	Treatment, g. or cc. per kg. of Soil	No. Pots	No. Infected	No. Plants	No. Infected	Initial pH	Final pH	Aver. pH
1.....	Sulfuric acid 3 cc.	5	3	7	4	4.3-4.5		4.4
2.....	Sulfuric acid 5 cc.	5	3	22	10	4.26-4.35	4.36-4.75	4.49
3.....	Sulfuric acid 10 cc.	5	0	28	0	3.66-3.79	4.00-4.10	3.91
4.....	Sulfuric acid 20 cc.	5	0	10*	0	2.84-2.96	3.25-3.79	3.17
5.....	Untreated control sets 1-7	5	3	12	5	6.60-6.78	6.59	6.59
6.....	Citric acid 10 g.	5	5	20	11	6.18-6.47	6.50-7.02	6.47
7.....	Citric acid 5 g.	5	5	6	6	6.0-6.5		
8.....	Acetic acid 5 g.	3	3	6	6	4.60-4.92	5.16-5.93	5.14
11a....	Sulfurous acid 1 g.	2	1	6	2	3.93-4.06	4.22-4.36	4.17
9.....	Inoculated sulfur 5 g.	5	0	16	0	3.79-3.90	4.00-4.23	3.92
10.....	Inoculated sulfur 3 g.	5	1	19	1	3.92-4.03	3.99-4.20	3.98
9a.....	Inoculated sulfur 0.75 g.	5	2	16	5	5.35-5.83	6.26	5.61
11.....	Inoculated sulfur 1.0 g.	5	1	17	3	4.03-4.21	4.52-4.97	4.30
9a-1....	Inoculated sulfur 1.0 g.	2	2	6	3	4.55-4.62	4.71-4.91	4.62
10a-1....	Inoculated sulfur 0.5 g.	2	0	8	0	3.94-4.02	3.72-3.79	3.87
9-1.....	Inoculated sulfur 0.25 g.	2	0	7	0	3.75-3.94	3.95-4.07	3.96
12.....	Inoculated sulfur 4 g. + citric acid 4 g.	5	4	18	6	4.51-4.68	4.88-5.18	4.82
13.....	CaCO <sub>3</sub> 10 g.	5	2	16	2	6.67-7.67	7.65-7.74	7.24
14.....	CaCO <sub>3</sub> 20 g.	5	1	12	2	7.18-7.54	7.54	7.40
15.....	CaCO <sub>3</sub> 30 g.	5	1	11	1	7.11-7.36	7.49	7.33
16.....	CaCO <sub>3</sub> 30 g.	2	1	2	1	7.36-7.37		7.36
17.....	Untreated control sets 9-15	4	4	11	6	5.53-6.61	6.67	6.31
13a.....	Ca(OH) <sub>2</sub> 1 g.	2	2	6	2	7.04-7.42	7.20-7.71	7.21
17a.....	Ca(OH) <sub>2</sub> 2 g.	3	2	9	3	6.83-7.20	7.05-7.10	7.02
5a.....	Ca(OH) <sub>2</sub> 4 g.	3	2	6	2	6.23-6.91	7.27-7.51	6.95
18.....	Ca(OH) <sub>2</sub> 30 g.	2	0	1	0		8.65-9.07	8.86
14a.....	CaO 2 g.	3	1	9	2	7.46-7.80	7.53-7.66	7.63
15a.....	CaO 4 g.	3	1	11	1	7.77-8.15	7.35-8.03	7.89
19.....	CaO 8 g.	2	1	6	1	8.42-8.83	7.81-8.26	8.17
20.....	CaO 10 g.	2	1	7	2	9.05-9.10	7.54-8.44	8.27
21.....	CaO 10 g.	2	0	2	0	8.20-8.36		8.28
22.....	CaO 15 g.	2	0	6	0	8.65-8.70	8.32-8.33	8.50
23.....	CaO 20 g.	2	0	8	0	9.00-9.55	8.45-8.59	8.90
24.....	CaO 20 g.	2	0	1	0	8.25-8.40		8.33
25.....	CaO 30 g.	2	0	1	0	12.06-12.10		12.08

TABLE 15 (Continued)

	Treatment, g. or cc. per kg. of Soil	No. Pots	No. Infected	No. Plants	No. Infected	Initial pH	Final pH	Aver. pH
	Untreated control sets 16, 18, 21, 24, 25	2	2	2	2	6.35-6.67		6.51
26.....	Soda-lime 3 g.	4	4	12	5	7.2-7.4	7.02-7.14	7.13
27.....	(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> 3 g.	4	2	7	3			
28.....	K <sub>2</sub> CO <sub>3</sub> 3 g.	3	3	6	4	7.52-7.80	7.06-7.42	7.46
29.....	NaOH 3 g.	3	2	12	2	8.01-8.64	7.35-8.06	8.11
30.....	Na <sub>2</sub> SO <sub>4</sub> 10 g.	2	2	4	3	6.33-6.41	7.08-7.34	6.79
31.....	MgNO <sub>3</sub> 5 g. CaSO <sub>4</sub> 10 g.	3	3	10	5	6.06-6.55	6.51-6.66	6.52
32.....	NaHPO <sub>4</sub> 5 g.	3	3	8	6	7.18-7.48	7.01-7.50	7.29
33.....	Untreated control sets 28 to 32	2	2	6	4	6.15-6.35	6.52-7.35	6.59
34.....	NaNO <sub>3</sub> 10 g.	3	3	10	3	6.48-7.05	7.15-7.67	7.09
	Untreated control	1	1	4	3	6.39	7.67	7.03
35.....	NaNO <sub>3</sub> 5 g.	3	3	8	3	6.84-6.92	7.03-7.32	7.06
	Untreated control	1	1	2	1	6.40	6.86	6.63
36.....	Fertilizer mixture 1, 1 g.	5	5	15	13	6.09-6.43	6.21-6.49	6.31
37.....	2 g.	5	4	13	5	5.88-6.03	5.84-6.12	5.93
38.....	Fertilizer mixture 2, 1 g.	5	5	16	8	6.11-6.34	6.28-6.62	6.34
39.....	2 g.	5	5	14	5	5.26-5.89	5.85-6.38	5.82
40.....	Fertilizer mixture 3, 1 g.	4	4	13	10	5.99-6.13	6.10-6.23	6.12
41.....	2 g.	5	5	24	17	5.05-5.48	5.72-6.75	5.74
42.....	Untreated control sets 36 to 41	3	1	8	3	5.39	7.81	
43.....	Field soil, untreated	3	3	8	5	5.05-5.32	5.00-5.71	5.26
44.....	Field soil from plots treated with sulfur, 400 lbs. per acre	3	3	6	5	4.23-4.51	4.70-4.88	4.56
45.....	Field soil + KHSO <sub>4</sub> 3 g.	3	3	6	4	4.28-4.48	4.90-5.14	4.72
46.....	Field soil + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> 3 g.	3	3	6	4	4.20-5.06	4.10-4.52	4.41
47.....	Field soil + NaNO <sub>3</sub> 3 g.	3	3	6	4	5.11-5.54	5.19-5.48	5.32
48.....	Semesan dry 1.25 g.	1	1	1	1	6.90		
	3.75 g.	1	0	1	0	8.11		
	6.25 g.	1	0	1	0	8.45		

#### THE BEARING OF THE FOREGOING CONCLUSIONS ON THE GEOGRAPHICAL DISTRIBUTION OF WART IN RELATION TO POTATO CULTURE

The requirements for infection as determined experimentally in respect to temperature of soil and air, soil-moisture content, and soil reaction correspond closely to the normal values of these factors in the wart-infested area around Freeland. Thus, infection is dependent on a mean temperature not exceeding 20°-22° C., and is favored by a somewhat acid soil reaction, by abundant rainfall, and by good drainage. From the latter part of May to the end of September meteorological conditions in this locality are usually favorable for the development and spread of the disease. In such respects as are susceptible of representation by climatological data it appears (table 16) that Freeland does not differ greatly from other places where wart occurs. The mean temperature during most of the growing season is somewhat nearer the upper limit for infection than at any of the

TABLE 16. *Meteorological Data for Selected Stations for Occurrence of Potato Wart*\*

Station	Temperature ° F.												Precipitation					
	Air						Soil 2-3 in.						Soil 6 in.			Total, Inches		
	June			July			June			July			June			July		
	Aug.	Sept.	June	July	Aug.	Sept.	June	July	Aug.	Sept.	June	July	Aug.	Sept.	June	July	Aug.	Sept.
Drifton, Pa.†	64.2	68.5	66.3	59.3	66.2	70.2	67.5	65.4	61.0	61.0	61.0	61.0	4.18	4.51	5.11	3.60	12	10
Thomas, W. Va.	67.5	70.3	56.1	60.0	60.6	54.0	62.0	67.1	65.0	61.0	61.0	61.0	4.89	5.38	4.74	3.60	9	13
St. John's, Nfld.	51.9	56.1	60.6	54.0	59.5	56.5	62	64	63	59	59	59	3.50	3.83	2.62	4.25	13	12
South Wales and S. W. England	57.5	60.0	59.5	56.5	62	64	63	59	60	56	56	56	1.4	3.0	2.7	3.3	9	16
East Scotland	53.8	56.3	55.5	50.1	59	61	60	56	60	56	56	56	1.2	2.7	3.5	2.7	13	16
Groningen, Holland	58.7	61.4	61.0	56.5	56.5	64.7	59.5	56.5	61.0	60.5	54.5	54.5	3.3	3.3	4.3	5.1	17	17
Cologne, Germany	62.7	65.7	64.7	59.5	56.5	61.8	56.0	56.0	56.5	61.0	60.5	54.5	3.3	3.3	4.3	5.1	17	17
Kiel, Germany ‡	59.0	62.5	61.8	56.0	56.0	61.8	56.0	56.0	56.5	61.0	60.5	54.5	3.3	3.3	4.3	5.1	17	17
Mandel, Norway	55.0	60.3	57.7	53.5	53.5	53.5	53.5	53.5	53.5	53.5	53.5	53.5	3.3	3.3	4.3	5.1	17	17

\* The data for Drifton and Freeland are from Weather Bureau or thermograph records as previously described; this applies to Thomas, W. Va., also. The sources of the other data are: St. John's, annual reports of the Minister of Agriculture, 1910-1920; English stations, British Meteorological Yearbook, 35-year record; Germany, Deutsches Meteorologisches Jahrbuch, 35-50 years; Norway, Jahrbuch des Norwegischen Meteorologischen Instituts, 1913-1918.

† Soil temperature data for Freeland, Pa.

‡ Soil temperature data for Bremen.

other stations, if an exception be made of Thomas, West Virginia. At both stations there is almost daily recurrence of temperatures below the thermal optimum for wart infection, *i.e.*, about 58° F., thus counteracting the lower mean, but more equable, temperature of the British Isles and of the maritime parts of continental Europe.

There is an appearance of heavier precipitation at the American stations, but this does not necessarily result in more soil moisture, as the rainfall is often excessive for short periods, causing a large amount of run-off. Although potatoes may withstand for a time more droughty conditions than are favorable to wart infection, so that moisture becomes a limiting factor for the disease but not for the host, deficiency of rainfall is seldom so great as to preclude at least late-season infection. In southern England in 1921, precipitation of only five inches all summer, but falling mostly late in August, caused infection. Brierley (6) ascribed the suppression of infection during summer largely to temperature. However, at Freeland, where the mean monthly temperatures in 1921 were June, 68° F., July, 74°, August, 65°, as compared with 59°, 66°, and 65°, respectively, for the midland counties in England, there was about the normal amount of wart infection. For the same months the rainfall at Freeland was 4.6, 7.3, and 4.5 inches, compared with 0.3, 0.7, and 3.5 inches at the British station.

Infection is therefore dependent on a certain balance of temperature and moisture conditions rather than on any isolated factor. This balance finds its integrated expression in vigorous growth of the host.

Orton (29) pointed out that, except for localities at high altitudes, Aroostook County, Maine, is the only potato section in the eastern United States which lies below the July isotherm of 65°. Smith (46) concluded, from a study of the effect of weather on the yield of potatoes, that the late-potato crop of the United States is practically limited to regions having a mean July temperature not in excess of 70° F., and that the greatest yields are obtained where the July mean is about 65°. June and July are the critical months for growth of this crop, more particularly the twenty-day period from June 21 to July 10, which Smith states should be "wet as well as cool." Field observations show that within this same period the initiation of wart infections and development of the overgrowths also are most rapid. An extensive comparison of climatological data for most of the important potato sections of the United States shows that, during the actual growing season for potatoes, temperature and rainfall do not depart widely from the conditions under which wart develops in Pennsylvania.

As compared with other potato diseases, wart is favored by conditions intermediate between those under which powdery scab and blackleg (34) on the one hand, and common scab (19, 38) on the other, are prevalent. There is a close parallel in the optimum conditions for late blight (26) and wart, as the thermal range and the optimum temperature for spore-germination are nearly the same. Similarly, the temperature at which tissue-

destruction by the late-blight fungus is most rapid nearly coincides with the optimum for shoot growth of the potato, and thus for wart overgrowths. So far as influenced by environmental factors, the distribution of these two potato diseases conceivably might coincide.

However, late blight is almost co-extensive with late-potato production in Europe and much of North America. Wart remains a disease of small gardens. The difference must be sought principally in the means by which the pathogens are disseminated, and the marked varietal differences in reaction to wart contrasted with general lack of resistance to blight. The conidia of *Phytophthora*, in consequence of their aërial situation, are readily disseminated; they may germinate either by liberation of zoöspores or by a germ tube, the latter method affording an extension of the thermal range for infection. The zoöspores seem to have a somewhat longer independent existence (26), and the extent of host tissue which potentially may be invaded is relatively vast.

On the other hand, the sporangia of *Synchytrium* are but slowly disseminated by soil movement and water currents; access to susceptible host parts, which are relatively few and of small extent, is difficult, and failure to gain it is rapidly fatal. These limitations on its dissemination and its strictly parasitic mode of life render it a much less aggressive parasite, and because of its controllability through seed-selection and cultivation of immune varieties, potato wart offers no serious problem where adequate quarantines can be enforced and suitable immune varieties are available.

#### SUMMARY

Infection by *Synchytrium endobioticum* is dependent on the presence of particular varieties of its host and on environmental conditions in general favorable to vigorous growth of the potato plant.

Germination of both resting and soral sporangia occurs in water, and there is an indispensable minimum of water for the distribution of the motile cells. If the soil-moisture content does not at any time reach saturation, germination is prevented, but if it is constantly near saturation, infection is repressed, probably through the reaction on the host. The most favorable condition is periodic flooding, followed by drainage and aëration. Infection may occur, if the temperature is favorable, in soil that is wet at insufficient intervals to afford a normal crop.

The complete thermal range for germination of resting sporangia was not determined, but infection resulted when they germinated between 10° and 28° C. Infection from germinating soral sporangia occurred between nearly 0° and 30° C. When the soil temperature was constantly maintained, infection was limited to the range 12° to 24° C., but with variable soil temperature, as in the field, infection occurs when the mean is about 21°, though the upper range may be as high as 30° C.

The most favorable soil reaction is from neutral to slightly acid, the



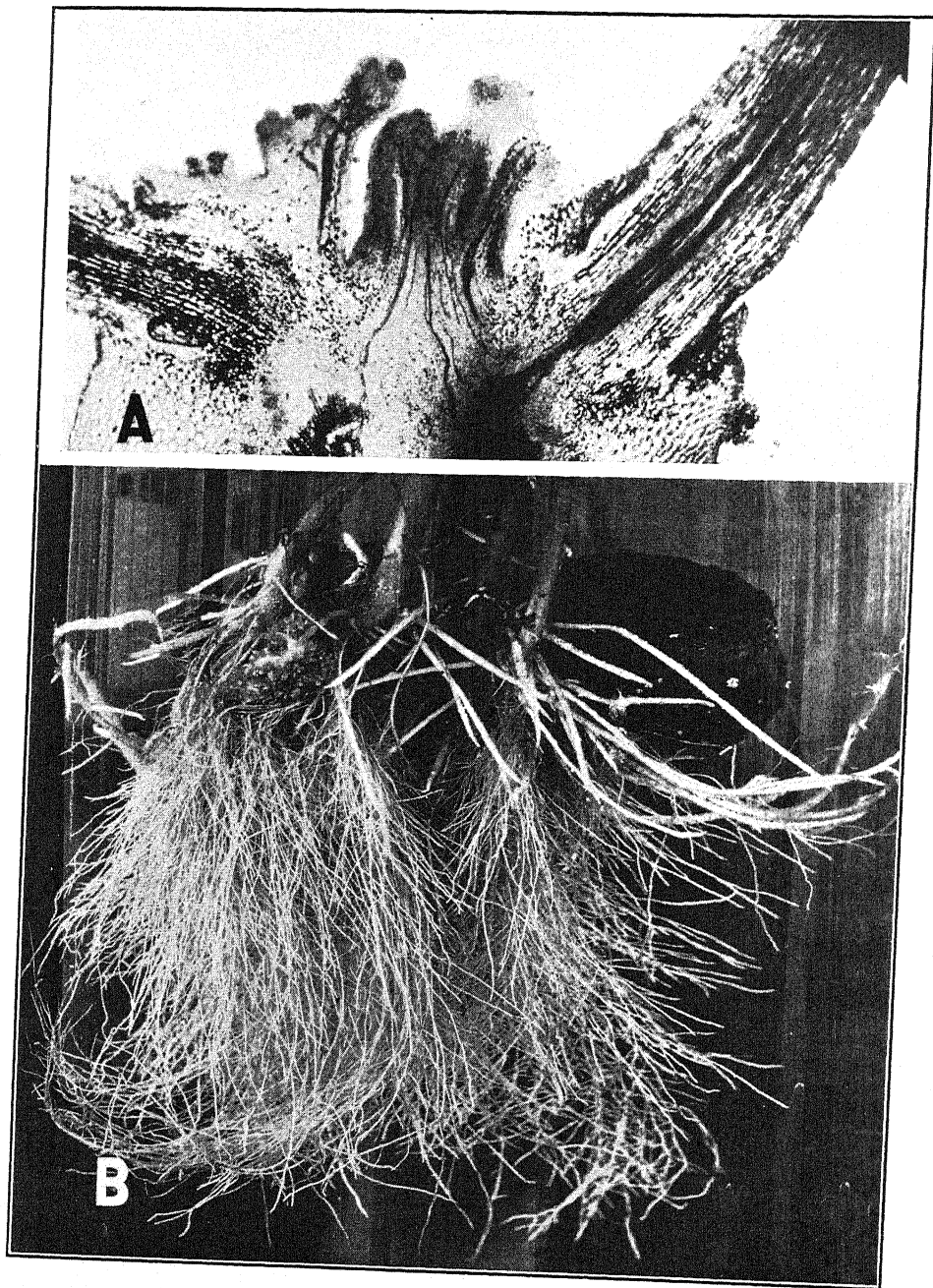
range being from about pH 3.9 to pH 8.5. The potato tolerates somewhat greater alkalinity but with reduction of yield and injury from other diseases.

Although the wart fungus and the potato plant have similar requirements as to environmental factors, the disease can not spread widely under an effective quarantine on the movement of infected seed. Its controllability through the use of immune varieties reduces it, in the United States, to a problem for which the solution is at hand.

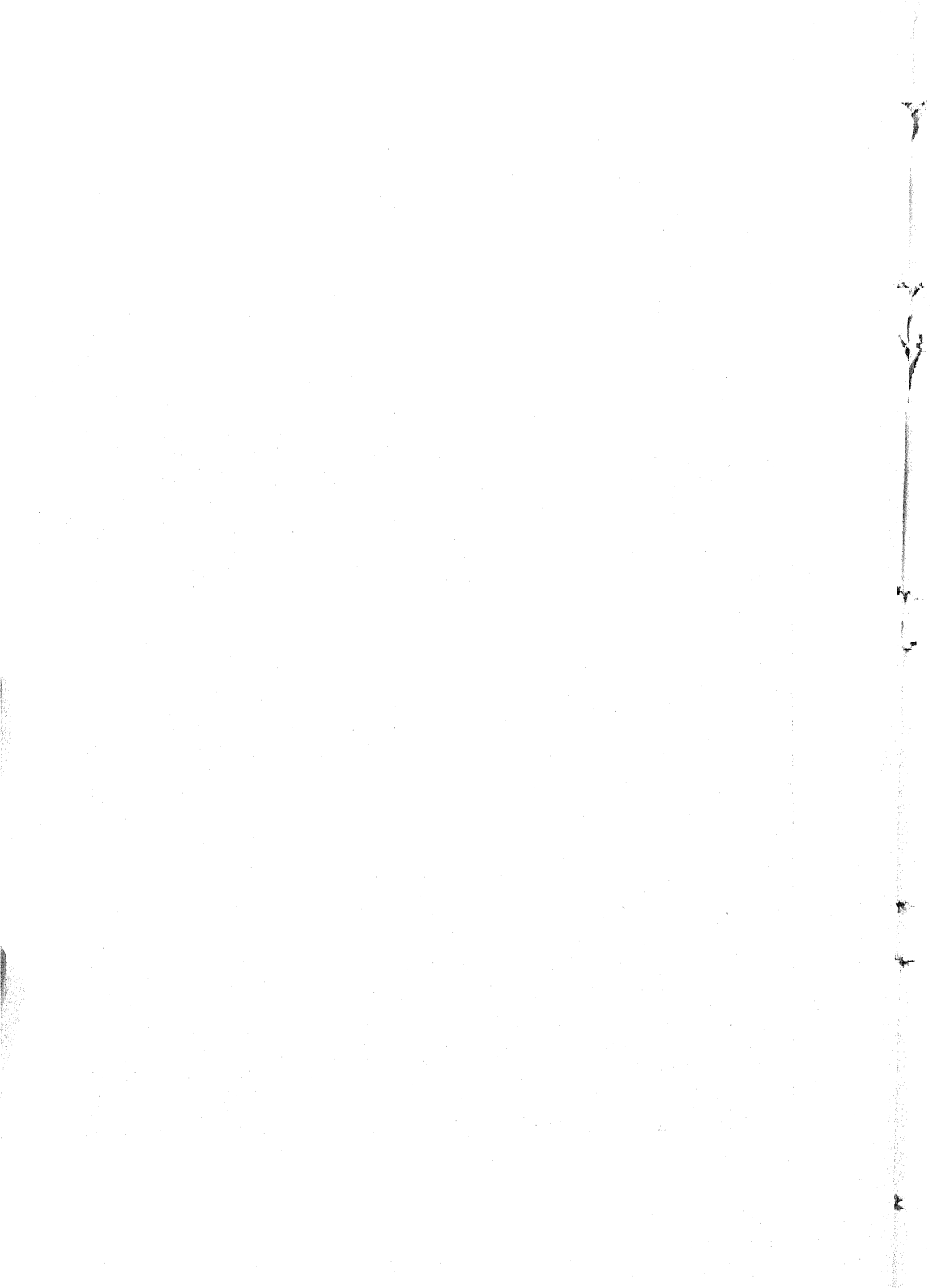
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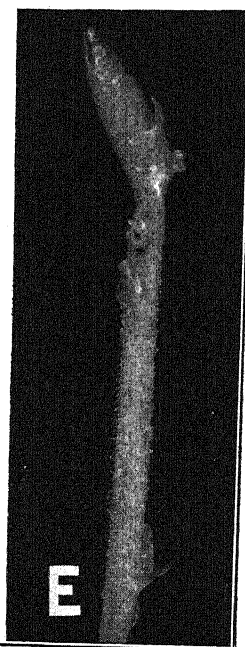
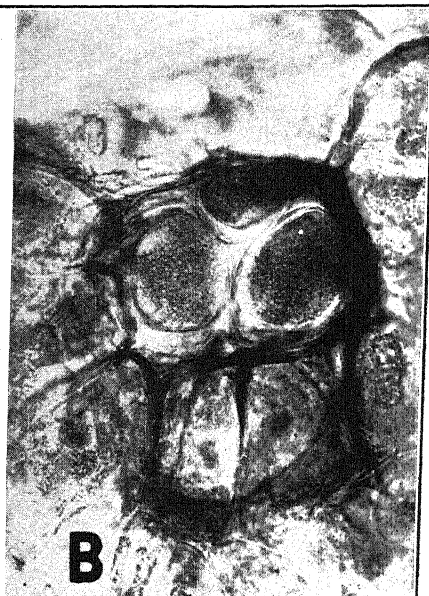
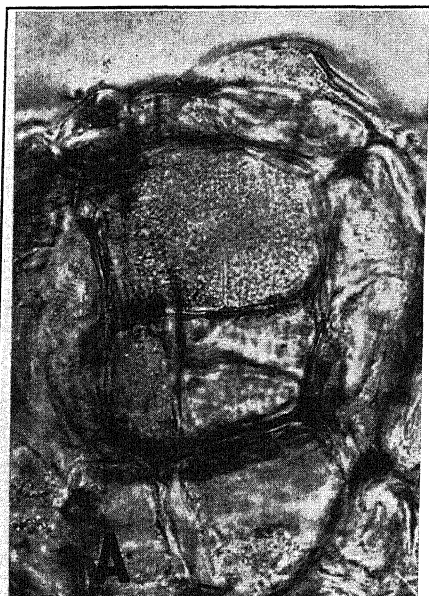
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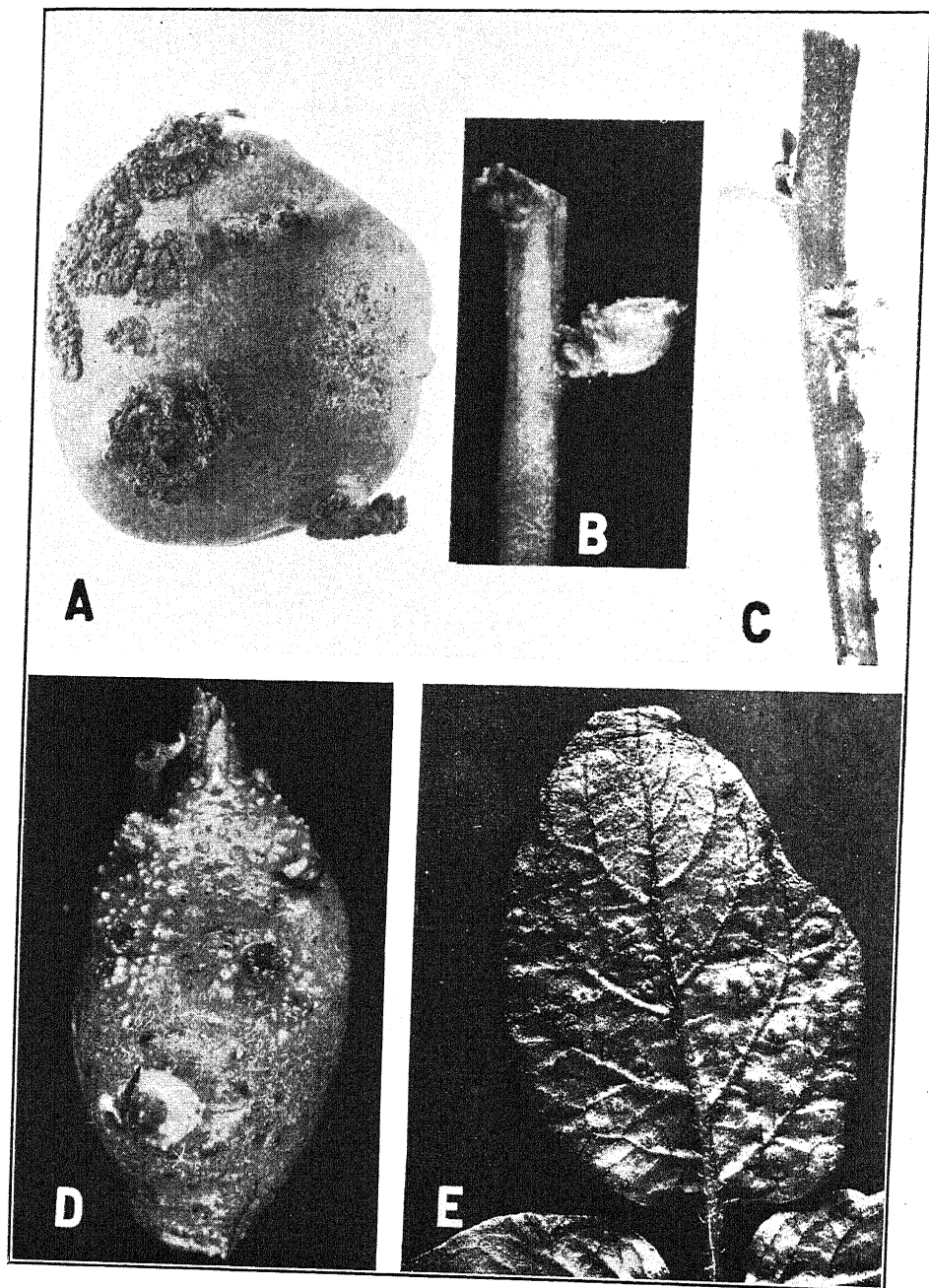
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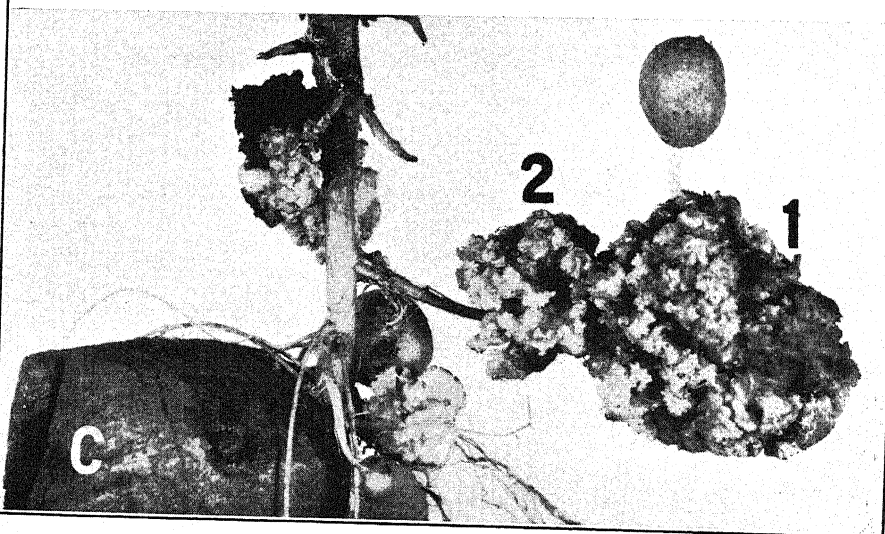
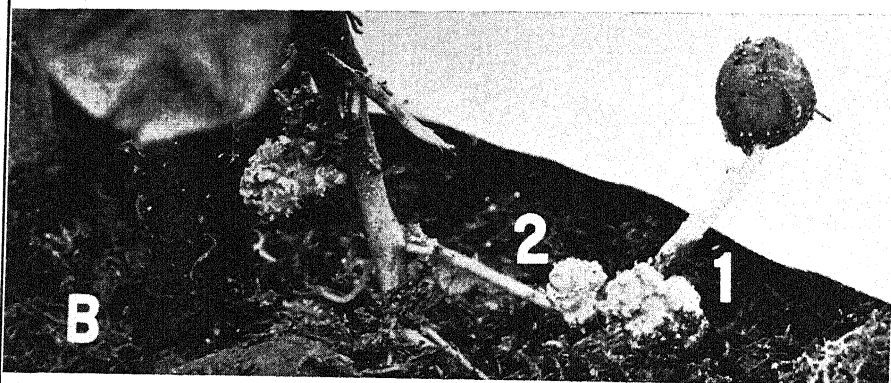
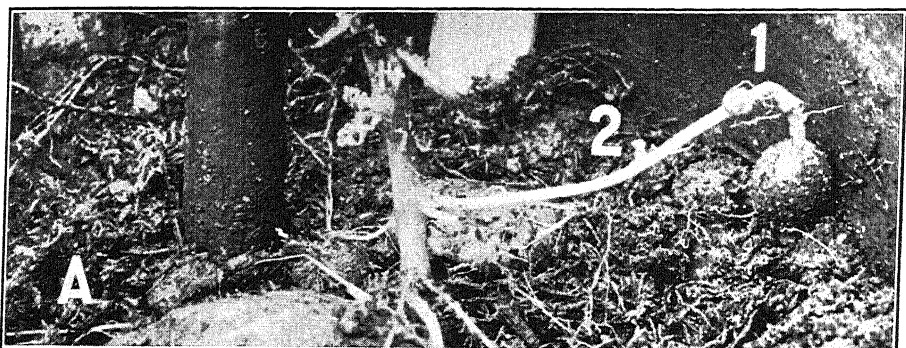




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## EXPLANATION OF FIGURES

### PLATE XLI

FIG. A. Section through base of stem showing emergence of 2 roots at left and right, and a stolon in the center. Sori of *Synchytrium* occur in tissue adjacent to but not within roots.  $\times 15$ .

FIG. B. Basal portion of potato plant in solution culture. Stolons and stem base bear wart infections but the roots are free.

### PLATE XLII

FIG. A. Early stage of cleavage in the sorus. Fresh unstained preparation.  $\times 400$ .

FIG. B. Mature intact sorus of sporangia. Soral membrane evident. Like A,  $\times 400$ .

FIG. C. Ruptured sorus with one remaining sporangium. Like A,  $\times 400$ .

FIG. E. Two sori on young stolon, infection occurring apart from buds.  $\times 4$ . Hypertrophy limited as compared with figure B, Plate XLIII.

### PLATE XLIII

FIG. A. Surface of tuber infected apart from eyes, producing a scab-like crust.

FIG. B. Infection of stolon bud and young tuber with formation of resting sporangia and production of considerable hypertrophy.  $\times 4$ .

FIG. C. Infection of internode of stem giving rise to small leaf-like excrescences.

FIG. D. Infection of young tuber, showing 6 sori but only local hypertrophy.  $\times 15$ .

FIG. E. Infection of potato leaf, showing numerous epidermal hypertrophies, mostly associated with sori, but resting spores also present.

### PLATE XLIV

FIGS. A, B, C. Stages in development of wart overgrowths. A, 3 days after inoculation; B, 6 days, and C, 30 days after inoculation.

# PHYSIOLOGICAL STUDIES ON THE GENUS PHYTOPHTHORA<sup>1</sup>

LEON H. LEONIAN

(Received for publication April 17, 1925)<sup>2</sup>

The specific distinctions within the genus *Phytophthora* are very limited, since most of its members exhibit a remarkable uniformity of morphological features. The taxonomy of the group, nevertheless, has been based largely upon the size and the shape of sporangia, the presence or the absence of oögonia, and the nature of the aërial and the submerged hyphae. Since these characteristics are extremely variable, no trustworthy specific segregation is possible unless a more stable basis of classification can be established. Certain physiological features, as manifested on solid agars, and the host relationships of these parasites have been employed to overcome the uncertainty of a purely morphological classification. However, neither such restricted physiological reactions nor most of the host relationships are capable of furnishing a satisfactory scheme of classification. A more intensive study of the physiology of *Phytophthoras* was found necessary, therefore, to establish dependable specific reactions and thus to render the taxonomy of this genus less difficult and more reliable.

A definite attempt has been made to conduct as many experiments as time and facilities would permit. One naturally expects to find a very large number of more or less nearly identical physiological reactions among the members of a genus which possesses such few and indefinite specific characteristics. Consequently, if the experiments had not been of sufficient diversity, the positive and negative specific reactions which have been tabulated in the following pages would not have been varied enough to separate the numerous species of this group.

## ORGANISMS

An attempt was made to bring together pure cultures of all the described species of *Phytophthora*, but this proved to be unsuccessful. *P. allii* Saw., *P. jatrophae* Jens., *P. melongenae* Saw., *P. meadii* McRae, *P. thalictri* Wils. & Davis, *P. theobromae* Colem., *P. fici* Rau., and *P. citri* Rau. were not available from any source. A request for a subculture of *P. allii* from Japan was not even answered. It is quite possible, however, that most, if not all, of the just named organisms are not specifically distinct. *P. meadii* differs from *P. faberi* chiefly by the fact that it gives rise to oögonia. Gadd (1)

<sup>1</sup> Published by the permission of the Director of the West Virginia Agricultural Experiment Station as scientific paper no. 11.

<sup>2</sup> Published, at the expense of the West Virginia Agricultural Experiment Station, out of the order determined by the date of receipt of the manuscript.

has, however, demonstrated heterothallism in *P. faberi* and has obtained perfect oögonia in pure cultures. He concludes that the supposed morphological differences between *P. meadii* and *P. faberi* have thus been reduced considerably. *P. thalictri* has been studied only imperfectly, and, unless it is worked out in detail and demonstrated to be a genuine species rather than a form of *P. infestans* (Mont.) DeBary or of *P. phaseoli* Thaxt., it may be disregarded. *P. fici* and *P. citri* have been named only provisionally and have been kept out of consideration. *P. theobromae* may be the same as *P. faberi*, as Lafferty and Pethybridge (2) seem to think. And lastly, *P. jatrophae* may prove to be a strain of *P. parasitica* Dast. or of *P. faberi*.

With the exception of the forms above mentioned, all the known species of Phytophthora and some new ones have been secured for these studies. Whenever possible, more than one form has been used. *P. parasitica*, *P. faberi*, and *P. palmivora* Butl. have a large representation of strains from widely separated localities and hosts. This fact has served as a very good check in the proper interpretation of experimental data. Some of the fifty-three organisms listed in the following pages arose as saltations and were separated in pure cultures. Their presence in the experiments described below indicates the probable taxonomic value of such forms and may counteract the natural enthusiasm for making new species with which the investigator is afflicted when confronted by the interesting phenomenon of saltation.

The following is a list of the organisms:

*P. arecae* (Colem.) Pethyb. Sent by G. Gundu Rao, Bangalore, India, upon the request of William McRae of the Pusa Station. Another culture was sent directly by McRae.

*P. cactorum* 1 (Lebert & Cohn) Schrot. Subculture of this organism was received from C. D. Sherbakoff, who obtained it from Holland.

*P. cactorum* 2. This is Beach's organism causing rhubarb rot. Received from Sherbakoff.

*P. cactorum* 3. Collected on the apple fruit by A. Berg at Raymond City, W. Va. Isolated by the writer.

*P. fagi* (Hartig) Hartig. Sent by Sherbakoff, who received it from Holland.

*P. capsici* Leonian. Isolated from peppers in New Mexico by the writer.

*P. citrophthora* (*Pythiacystis citrophthora* Sm. & Sm.). Obtained from H. S. Fawcett. This organism is so obviously a Phytophthora species that the genus *Pythiacystis* is no longer tenable.

*P. cinnamoni* Rands. Sent by R. D. Rands.

*P. cryptogea* Pethyb. & Laf. Subculture furnished by Frank B. Cotner. The original came from Holland.

*P. mexicana* Hotson & Hartge. Received from J. W. Hotson.

*P. erythroseptica* Pethyb. Obtained from Sherbakoff, who received it from Holland.

*P. infestans* (Mont.) DeBary. Furnished by A. Berg.

*P. phaseoli* Thaxt. Isolated from navy beans by the writer at Morgantown, W. Va.

*P. nicotianae* Van Breda de Haan. Received from C. D. Sherbakoff, who obtained it from Holland.

*P. syringae* (Klebh.) Klebh. Obtained from Holland.

*P. parasitica* Dastur. Sent by Pethybridge. Another culture was obtained from

R. J. Tabor. These cultures originally came from Dastur.

*P. terrestris* Sherb. Obtained from Sherbakoff.

*P. sp.* (Reddick's) I. Received from F. B. Cotner, who obtained it from Reddick.

*P. sp.* (Reddick's) II. Saltant of *P. sp.* (Reddick's) I. Separated from a petri-dish colony by the writer.

*P. parasitica-rhei* I. Godfrey. Received from G. H. Godfrey.

The following four forms arose as saltations from *P. parasitica-rhei* and were separated and isolated in pure cultures by the writer:

*P. parasitica-rhei* II.

*P. parasitica-rhei* III.

*P. parasitica-rhei* IV.

*P. parasitica-rhei* V.

*P. faberi* Maub. Sent by Charles Drechsler, who received it from S. F. Ashby of the West Indian Agricultural College, Trinidad. Isolated from cacao pod.

*P. palmivora* (Butl.) Butl. Sent by Pethybridge, who received the original culture from E. P. Butler. The organism was isolated by Ashby from cacao fruit in the West Indies.

*P. colocasiae* Rac. Sent by W. McRae of Pusa, India.

The following organisms were sent by Carl Hartley. They represent his and Reinking's collection of tropical Phytophthoras. The numbers used in connection with the organisms are Hartley's, and are retained throughout this work until the conclusions are given; then a proper identification, based exclusively upon the physiological behavior of these organisms, is made.

No. 145. From cacao, Surinam. Received from Holland.

No. 143. From Abaca; isolated by Reinking in the Philippines.

No. 139. From eggplant; isolated by Reinking in the Philippines.

No. 126. From coconut; isolated by Reinking in the Philippines.

No. 136. Hartley lists this organism as merely a subculture of no. 126. It seems to be a distinct saltation of no. 126, which saltates persistently on malt-extract agar.

No. 138. From eggplant; isolated by Reinking in the Philippines.

No. 142. From Hibiscus; isolated by Reinking in the Philippines.

No. 140. From cacao; isolated by Reinking in the Philippines.

No. 127. Isolated by Welles in the Philippines.

No. 137. Hartley states that this organism is "perhaps the same strain as no. 127."

No. 123. From Borassus in India; labelled "*P. palmivora*."

No. 144. From Citrus; isolated by Reinking in the Philippines.

No. 117. From Hevea twigs; isolated by Arens in East Java.

No. 102. From Hevea; isolated by Vischer in Buitenzorg.

No. 22. From cacao pod; isolated by Hartley in Java.

No. 26. From cacao pod; isolated by Hartley in Java.

No. 97. From Erythrina; isolated by Hartley in Buitenzorg.

No. 116. From Hevea fruit; isolated by Arens in Java.

No. 100. From cacao cankers; isolated by Hartley in Java.

No. 141. From Hevea seedlings; isolated by Reinking in the Philippines.

No. 8. From cacao pod; isolated by Hartley in Java.

No. 121. From cacao cankers; isolated by Hartley in Buitenzorg.

No. 44. From cacao pod; isolated by Hartley in Buitenzorg.

No. 36. From cacao canker; isolated by Hartley in Buitenzorg.

No. 372. Isolated from the roots of *Pinus resinosa* in Minnesota by Roy G. Pierce and sent to the writer by Annie R. Gravatt.

The writer wishes to extend his hearty thanks and deep appreciation to all his colleagues for their friendly coöperation throughout the course of this work.

#### TECHNIQUE

Two solid media and one nutrient solution have been used throughout this work. The formulae for the solid agars are as follows:

##### No. 1. Malt-extract Agar

Malt extract (dry).....	5 g.
Dihydrogen potassium phosphate.....	0.6 g.
Magnesium sulfate.....	0.3 g.
Bacto agar.....	20 g.
Distilled water.....	1,000 cc.

##### No. 2. Nucleinic-acid Agar

Nucleinic acid.....	1 g.
Dihydrogen potassium phosphate.....	0.6 g.
Magnesium sulfate.....	0.3 g.
Dextrose.....	3.6 g.
Bacto agar.....	20 g.
Distilled water.....	1,000 cc.

The ingredients were heated together in the autoclave at seven pounds' pressure for ten minutes, filtered through absorbent cotton, tubed, and sterilized at ten pounds' pressure for fifteen minutes.

The formula for the nutrient solution is the same as that for medium no. 2, except that no agar is used. The ingredients were added to 200 cc. of distilled water and heated over an open flame. After boiling for a minute the solution was filtered through two thicknesses of filter paper; sufficient distilled water was then added to bring the volume to 1000 cc. It was then sterilized at ten pounds' pressure for fifteen minutes.

The technique used with the solid agars requires no further explanation, except that whenever test-tube cultures were used 10 cc. of the agar was poured into each tube (tubes being 15 mm. by 145 mm.). In the case of petri-dish cultures 20 cc. of agar was poured into each dish (plates being 10 mm. by 90 mm.). The temperature, unless otherwise stated, was uniform (20° C.) throughout this work.

A more detailed explanation is necessary in case of the technique employed in the liquid-culture experiments. Only one type of dish was used; it has been termed a glass capsule, and is listed in the catalogs of scientific apparatus as a "preparation dish." It is of 40-cc. capacity, has a loosely fitting cover, and is 30 mm. deep with a width of 45 mm. at its base, and 40 mm. at its top.

Stock cultures for solid-agar work were carried in oatmeal agar, while those for liquid cultures were kept in nucleinic-acid solution in capsules.

Approximately 25 cc. of the nutrient solution was poured into a sterilized capsule, and then a bit of mycelium was transferred from a tube to this solution. Except in the cases of *P. infestans* and *P. phaseoli*, all the organisms grew very rapidly in this solution and formed a large amount of mycelium within a few days. In a number of cases the sporangia, when transferred from agar tubes to the nutrient solution, formed a large number of zoöspores, each of which settled to the bottom of the dish and there grew into a distinct colony. *P. infestans* required a week or ten days to produce colonies large enough to use for making transfers. *P. phaseoli* grew still more slowly in this medium, but when a few cc. of lima bean infusion was added (2 g. of green lima beans, 100 cc. of water), this organism grew very readily. *P. phaseoli* was, therefore, grown in nutrient solution to which a few cc. of bean infusion had been added; all others were grown in the pure solution.

The stock cultures in the capsules were renewed every two weeks, or oftener. In addition to the elimination of the danger of contaminated cultures, this procedure assured a vigorous organism for the propagation of the experimental colonies.

After a good mycelial growth had been formed in the stock dishes, the culture work was begun. Twenty-five cc. of nutrient solution was poured into each of a series of capsules, and with the aid of fine-pointed forceps, sterilized in flame, six very minute bits of hyphae were taken from the stock cultures and transferred to each dish. Care was taken to make the inoculum just as small as possible. The purpose of this precaution was to avoid the introduction of large bits of mycelium from an old culture which was likely to have produced a number of sporangia. The chief objective of the experimental work outlined here is a vigorously growing *sterile* mycelium. Consequently, if the original inoculum is large, it is likely to prove misleading when the colony developed from it is examined under the microscope. The cultures thus made were kept at a temperature of 20–22° C. Three days later each bit of mycelium had formed a large colony of vigorously growing sterile hyphae. If allowed to remain longer than four or five days, or if grown in shallower solutions, or if growth was stimulated by incubation at higher temperatures, many of the organisms grew nearly to the surface of the solution and formed sporangia. The chief limiting factor in the reproduction of *Phytophthora* is access to atmospheric oxygen. If kept under a column of solution, the mycelium remains sterile although it grows readily. Eventually, however, the hyphae reach the surface and begin to form sporangia. Except when working with a slowly growing organism, therefore, no colonies in nutrient solution were allowed to grow longer than three days; then the vigorous but *sterile* mycelium was washed free from the nutrient solution by transferring it, with the aid of flamed forceps, to sterile distilled water in glass capsules. Fifteen minutes later it was transferred to 2 cc. of one of the various solutions mentioned in this work.



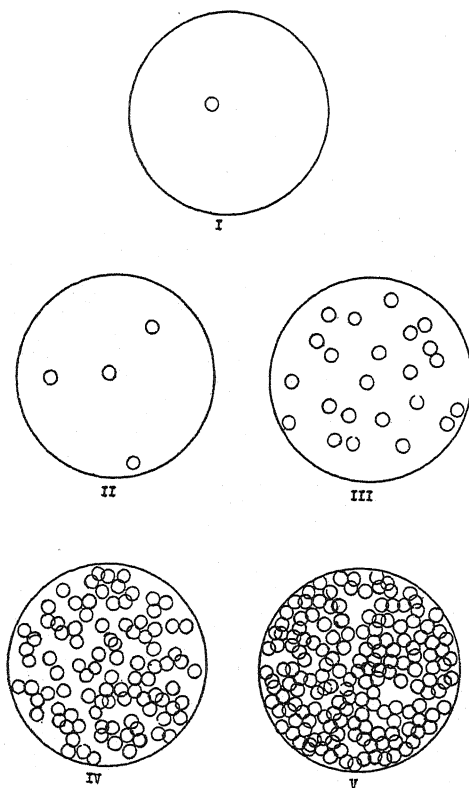
These solutions were placed in a series of sterile capsules by means of sterilized pipettes. Three days after the transfer the cultures were examined for sporangia and oögonia. The entire colony was examined, because the sporangial distribution is often uneven, or sometimes only a portion of the mycelium is fertile while the other is wholly or partially infertile.

All cultures were made in duplicate, and every one of the experiments, regardless of the nature of the results, was repeated once. Then all the negative results (indicated by the symbol o), and all the slightly positive results (indicated by the symbol I) were repeated three more times. Some were repeated eight or ten times, and no effort was spared to make the results as accurate and trustworthy as the somewhat peculiar physiology of this group allows. In spite of all efforts, however, the results given in this paper are not meant to represent the final analysis. They should be repeated by workers in different parts of the world and with different strains before a conclusive physiological classification can be perfected. Any one, therefore, who wishes to repeat these experiments, or who wishes to identify an unknown organism according to the physiological key given at the end of this paper, should bear in mind that a superficial, hasty, or limited effort will not yield satisfactory results; that one's technique should be perfected before the results become trustworthy; that one should be willing and eager to repeat the experiments with a given organism at least eight or ten times, and that a large number of cultures in each experiment should have been made before drawing conclusions. It should be remembered that the conditions in different parts of the country and of the world can never be standardized and controlled in the laboratory, that consequently certain variations may be exhibited by organisms, and that, unless fundamental differences are observed, one should hesitate a long time before forming conclusions. Finally, it should not be forgotten that the living protoplasm possesses a plastic nature which does not always adapt itself with clock-like regularity to all the environmental factors all the time.

*Symbols.* Roman numerals have been used as symbols to indicate the relative quantity of aërial mycelium, sporangia, and oögonia. When applied to the aërial hyphae, o indicates a total absence; I, very little, hardly noticeable; V, very abundant, the entire slant of the tube being filled with hyphae; IV, abundant, most of the slant being occupied with hyphae; III, fair, over one half of the slant covered with mycelium; II, poor, just the lower part of the slant showing some aërial hyphae, or just a few wefts scattered over the slant.

When applied to the presence and the absence of sporangia or oögonia, o indicates absolute sterility; I, very poor reproduction, perhaps only one or two sporangia in the entire colony; V indicates that the entire field is packed with sporangia or oögonia. The diagrams of text figure 1 represent the relative values of the symbols. The large circle represents the microscopic field (16-mm. objective and 10x ocular), and the smaller circles within the large one are the sporangia or oögonia.

While it is probable that the results indicated as 0 and I, I and II, II and III, III and IV, IV and V may merge, it is highly improbable that 0 and II, I and III, II and IV, and III and V will merge. In view of the fact that the specific reactions upon which the physiological segregation



TEXT FIG. 1. Relative value of the symbols used in the tables. The large circles represent the field of the microscope (low power); the smaller circles represent the reproductive bodies.

discussed in the following pages is based rarely represent anything closer than 0 and III or 0 and IV, the danger from the personal equation in the interpretation of the results and from the physiological fluctuations of the organisms has been reduced considerably. The physiological saltations, in consequence of which a number of organisms exhibit a negative reaction at one time and a positive reaction at another, have been recorded properly.

## THE EXPERIMENTS

### On Solid Agar

*Plate cultures.* The rate of growth of the colonies was the first point tested. Petri-dish plates of malt-extract agar, of concentrated malt-extract

agar (twenty times the normal concentration), and of nucleic-acid agar were inoculated with the organisms. The cultures which were to be subjected to the influence of the extreme temperatures (12° and 30° C.) were first incubated at 20° C. for one day and then transferred to their respective temperatures. The colonies were measured six days after inoculation.

All cultures were made in duplicate. Whenever there was too wide a margin of fluctuation in the size of the colonies, the cultures were repeated until a reasonable average was obtained. Most of the organisms, however, were fairly constant in their rate of growth. Nevertheless, the average measurements given in the following table are not at all emphasized in the final analysis of the reactions. If combined with the more pronounced and specific characteristics which are listed in the other tables, they may be of slight value. *P. infestans* and *P. phaseoli* alone show significant and specific reactions in table I.

TABLE I. *Diameter of Colonies Six Days after Inoculation*

Organisms	Malt-extract Agar			Concentrated Malt-extract Agar. 20° C. mm.	Nucleic-acid Agar. 20° C. mm.
	12° C. mm.	20° C. mm.	30° C. mm.		
<i>P. arecae</i> .....	0	60	80	45	55
<i>P. cactorum</i> 1.....	20	50	50	25	45
<i>P. cactorum</i> 2.....	19	50	55	30	50
<i>P. cactorum</i> 3.....	15	50	50	20	52
<i>P. fagi</i> .....	22	50	55	25	50
<i>P. capsici</i> .....	8	45	65	37	30
<i>P. citrophthora</i> .....	20	50	65	40	55
<i>P. cinnamoni</i> .....	15	55	52	30	60
<i>P. cryptogea</i> .....	35	60	68	45	67
<i>P. erythroseptica</i> .....	30	65	55	30	70
<i>P. mexicana</i> .....	20	62	80	27	60
<i>P. infestans</i> .....	0	0	0	0	0
<i>P. phaseoli</i> .....	0	0	0	0	0
<i>P. nicotianae</i> .....	10	40	52	22	40
<i>P. syringae</i> .....	23	45	0	20	47
<i>P. parasitica</i> .....	8	30	50	8	27
<i>P. terrestris</i> .....	13	35	53	20	30
<i>P. sp.</i> (Reddick's) I.....	11	40	65	8	37
<i>P. sp.</i> (Reddick's) II.....	10	70	65	35	60
<i>P. parasitica-rhei</i> I.....	16	40	55	25	40
<i>P. parasitica-rhei</i> II.....	8	20	50	20	42
<i>P. parasitica-rhei</i> III.....	5	35	55	10	37
<i>P. parasitica-rhei</i> IV.....	5	35	55	15	38
<i>P. parasitica-rhei</i> V.....	10	40	65	15	48
<i>P. faberi</i> .....	5	45	75	17	47

TABLE I (Continued)

Organisms	Malt-extract Agar			Concentrated Malt-extract Agar. 20° C. mm.	Nucleinic-acid Agar. 20° C. mm.
	12° C. mm.	20° C. mm.	30° C. mm.		
<i>P. palmivora</i> .....	5	50	85	25	47
<i>P. colocasiae</i> .....	12	45	68	20	45
139.....	10	37	55	20	27
142.....	10	40	55	15	27
143.....	12	38	56	17	37
145.....	10	43	50	18	35
123.....	10	55	85	22	60
126.....	30	60	82	45	40
136.....	7	48	70	25	40
127.....	8	50	80	17	47
137.....	12	70	85	22	60
138.....	10	75	90	40	70
140.....	5	45	78	20	58
144.....	12	41	60	15	42
22.....	10	68	82	35	55
26.....	8	50	72	20	48
97.....	13	65	100	50	65
100.....	12	50	65	37	52
102.....	5	65	83	25	48
117.....	5	55	55	25	50
116.....	10	70	88	35	60
141.....	17	45	62	18	50
8.....	20	67	80	35	50
36.....	30	63	80	35	60
44.....	25	70	82	40	70
121.....	15	60	82	35	65
130.....	25	60	80	45	65
372.....	45	85	90	40	100

The only specific reaction brought out in this table is the negative result manifested by *P. infestans* and *P. phaseoli*. These two organisms can be separated from all others by their growth in malt-extract and nucleinic-acid agars in petri dishes, since these are the only species which form no colony within six days at a temperature of 20° C.

The rate of growth of the colonies of the remaining organisms should never be considered a specific feature. There is too much similarity between the rates of growth of dissimilar organisms, and too much dissimilarity between those of similar organisms. *P. sp.* (Reddick's) II grows nearly twice as fast as *P. sp.* (Reddick's) I, yet essentially the two are the same fungus. Similarly, *P. parasitica-rhei* V grows twice as fast as *P. parasitica-rhei* II. *P. syringae* and *P. faberi* have the same rate of growth, but they represent very distinct organisms. And when we consider the fact that normal fluctuations vary from five to twenty millimeters, depending on whether the organism is a slow or a fast grower, the value of colony measurement becomes still less significant. If, however, all other reactions of a given organism are taken into consideration, the rate of growth may then become an additional, although very slight, means of identification.

Similarly, the type of growth of the colonies in petri dishes is not of primary importance. An examination of Plates XLV-XLVIII reveals the fact that many dissimilar organisms make similar growth and many similar organisms make dissimilar growth. *P. citrophthora* and no. 130 are very similar in their appearance and altogether unlike in their specificity. *P. arecae*, *P. cryptogea*, and *P. fagi* cannot readily be told apart, yet they are definite species. *P. parasitica-rhei* I does not at all look like *P. parasitica-rhei* III, and the latter is very different from *P. parasitica-rhei* V, while *P. parasitica-rhei* IV is very like *P. syringae*, or no. 116-II. Nevertheless, as cumulative evidence, the types of growth of the colonies may be of some value. Taken by themselves, however, or even when combined with only a limited number of reactions, they are likely to lead the investigator astray.

*Test-tube cultures.* Ten cubic centimeters each of the usual malt-extract agar, of the concentrated malt-extract agar (twenty times the normal concentration), and of nucleinic-acid agar were poured into a series of test

TABLE 2. *Aërial Hyphae*

Organisms	Malt-extract Agar	Nucleinic-acid Agar	Concentrated Malt-extract Agar
			Growth on Agar Slant
<i>P. arecae</i> .....	I	I	o Slightly wrinkled
<i>P. cactorum</i> 1.....	II	II	III Smooth
<i>P. cactorum</i> 2.....	I	II	III Smooth
<i>P. cactorum</i> 3.....	II	II	I Smooth
<i>P. fagi</i> .....	I	I	II Smooth with short pubescence
<i>P. capsici</i> .....	o	o	o Smooth
<i>P. citrophthora</i> .....	II	II	o Wrinkled
<i>P. cinnamomi</i> .....	II	III	o Fine, granular wrinkles
<i>P. cryptogea</i> .....	o	o	o Smooth
<i>P. erythroseptica</i> .....	II	III	III Smooth
<i>P. mexicana</i> .....	o	o	III Smooth
<i>P. infestans</i> .....	II	III	o No growth
<i>P. phaseoli</i> .....	II	o	o No growth
<i>P. nicotianae</i> .....	I	o	I Smooth
<i>P. syringae</i> .....	o	II	o Smooth
<i>P. parasitica</i> .....	III	III	o Deeply wrinkled
<i>P. terrestris</i> .....	III	III	o Very deeply wrinkled
<i>P. sp.</i> (Reddick's) I.....	III	III	o Deeply wrinkled
<i>P. sp.</i> (Reddick's) II.....	IV	III	III Slightly wrinkled
<i>P. parasitica-rhei</i> I.....	IV	III	II Slightly wrinkled
<i>P. parasitica-rhei</i> II.....	II	II	o Deeply wrinkled
<i>P. parasitica-rhei</i> III.....	o	o	o Deeply wrinkled
<i>P. parasitica-rhei</i> IV.....	o	o	II Wrinkled
<i>P. parasitica-rhei</i> V.....	II	I	II Slightly wrinkled

TABLE 2 (Continued)

Organisms	Malt-extract Agar	Nucleinic-acid Agar	Concentrated Malt-extract Agar
			Growth on Agar Slant
<i>P. faberi</i> .....	I	I	o Deeply wrinkled
<i>P. palmivora</i> .....	III	II	o Deeply wrinkled
<i>P. colocasiae</i> .....	II	I	II Smooth
139.....	II	III	I Deeply wrinkled
142.....	III	II	o Deeply wrinkled
143.....	IV	III	o Wrinkled
145.....	I	I	o Wrinkled
123.....	III	II	o Deeply wrinkled
126.....	III	I	III Smooth
136.....	I	I	o Wrinkled
127.....	II	II	o Smooth
137.....	I	I	o Smooth
138.....	III	IV	III Smooth
140.....	II	II	o Smooth
144.....	II	II	o Wrinkled
22.....	o	II	o Deeply wrinkled
26.....	II	II	III Smooth
97.....	II	II	I Deeply wrinkled
100.....	I	II	o Deeply wrinkled
102.....	o	I	o Deeply wrinkled
117.....	o	II	o Deeply wrinkled
116.....	III	I	o Smooth and glossy
141.....	I	I	o Wrinkled
8.....	I	I	I Smooth
36.....	I	o	III Smooth
44.....	II	o	III Smooth
121.....	II	I	III Smooth
130.....	I	o	III Smooth
372.....	I	II	I Smooth

tubes and sterilized. All transfers were made on the same day, and the cultures were kept at 20° C. One month later they were examined for aërial mycelium, sporangia, and oögonia. The results have been tabulated in tables 2-4.

Table 2 affords no justification for the practice of making the production of aërial hyphae a distinctly specific factor. The saltants of *P. parasitica-rhei* and of Reddick's *Phytophthora* exhibit numerous intergradations in the amount of aërial hyphae that may be produced by the strains of a single species. The twelve organisms listed in the table as nos. 139-144 belong to the same species, as will be seen later, but exhibit an extreme variation in the amount of aërial hyphae that they develop. It follows, therefore, that from the point of view of a common-sense classification neither table 1 nor table 2 offers any dependable specific factors. The differences which are brought out have no value except cumulatively in a system of taxonomy wherein a large number of fixed physiological reactions are used.

Table 3 illustrates still further the complexity of phases that may be

manifested by a given species, but it also tends to segregate certain well marked groups. *P. capsici*, *P. cinnamoni*, *P. cryptogea*, *P. erythroseptica*, *P. mexicana*, no. 116, nos. 8 and 372 show a remarkable lack of sporangium-production. *P. arecae*, *P. parasitica-rhei* IV, nos. 36, 44, and 130 can be included in the foregoing group without much difficulty, but *P. parasitica-rhei* IV is not at all fixed in its reactions and may at any time revert to *P. parasitica-rhei* I. Nos. 22, 97, 117, 141, and 121, when grown on malt-extract agar, introduce the phenomenon of physiological saltation. In spite of the fact that all the conditions have been absolutely alike, so far as they could be controlled in the laboratory, these organisms have manifested at one time a negative reaction, and at another time a positive reaction. This phenomenon will be still better illustrated a little later; suffice it to say that nos. 22, 26, 97, 100, 117, 116, and 141 on the one hand, and nos. 8, 36, 44, 121, and 130 on the other hand, represent a group of organisms

TABLE 3. *Sporangium-production (30 days at 20° C.)*

Organisms	Malt-extract Agar	Nucleinic-acid Agar	Concentrated Malt-extract Agar
<i>P. arecae</i> .....	I	I	o
<i>P. cactorum</i> 1.....	III	III	III
<i>P. cactorum</i> 2.....	III	III	II
<i>P. cactorum</i> 3.....	III	III	I
<i>P. fagi</i> .....	IV	IV	II
<i>P. capsici</i> .....	o	o	o
<i>P. citrophthora</i> .....	II	III	o
<i>P. cinnamoni</i> *.....	o	o	o
<i>P. cryptogea</i> .....	o	o	o
<i>P. erythroseptica</i> .....	o	o	o
<i>P. mexicana</i> .....	o	o	o
<i>P. infestans</i> .....	III	III	no growth
<i>P. phaseoli</i> .....	II	I	no growth
<i>P. nicotianae</i> .....	III	II	o
<i>P. syringae</i> .....	II	III	o
<i>P. parasitica</i> .....	IV	IV	o
<i>P. terrestris</i> .....	IV	IV	o
<i>P. sp. (Reddick's) I</i> .....	IV	IV	I
<i>P. sp. (Reddick's) II</i> .....	V	IV	II
<i>P. parasitica-rhei</i> I.....	IV	III	I
<i>P. parasitica-rhei</i> II.....	III	III	o
<i>P. parasitica-rhei</i> III.....	III	II	I
<i>P. parasitica-rhei</i> IV.....	I	I	I
<i>P. parasitica-rhei</i> V.....	III	III	II
<i>P. faberi</i> .....	III	IV	o

\* *P. cinnamoni* formed only a large number of chlamydospores in this as well as in the subsequent experiments. Except in this organism, sporangia and chlamydospores have not been differentiated from each other throughout this work.

TABLE 3 (Continued)

Organisms	Malt-extract Agar	Nucleinic-acid Agar	Concentrated Malt-extract Agar
<i>P. palmivora</i> .....	III	IV	II
<i>P. colocasiae</i> .....	II	II	o
139.....	III	IV	I
142.....	III	IV	I
143.....	III	IV	I
145.....	III	IV	o
123.....	IV	V	III
126.....	IV	IV	o
136.....	III	IV	o
127.....	V	IV	o
137.....	IV	V	o
138.....	V	V	III
140.....	III	IV	I
144.....	II	III	o
22.....	o-II	IV	o
26.....	o	III	o
97.....	o-II	III	o
100.....	o	III	o
102.....	o	III	o
117.....	o-III	III	o
116.....	o	o	o
141.....	o-II	II	o
8.....	o	o	o
36.....	II	I	o
44.....	o	I	o
121.....	o-II	I	o
130.....	o	I	o
372.....	o	o	o

which are oscillating between different forms. At one time they appear as one species, and at another time as a different one. The surprising part of it all is that two seemingly different species merge into each other, sometimes one species coming to the fore and sometimes the other; or both may appear at the same time and in the same culture.

TABLE 4. *Oögonium*-production (30 days at 20° C.)

Organisms	Malt-extract Agar	Nucleinic-acid Agar	Concentrated Malt-extract Agar
<i>P. cactorum</i> 1.....	III	III	II
<i>P. cactorum</i> 2.....	III	III	II
<i>P. cactorum</i> 3.....	II	III	o
<i>P. fagi</i> .....	II	I	III
<i>P. capsici</i> .....	I	o	o
372.....	IV	IV	II
<i>P. erythroseptica</i> .....	I	o	o

The third column of table 3 shows that it is not wise to generalize concerning what a group of organisms may and may not do. There seems to be a prevalent impression throughout the literature that *Phytophthoras*



and the related organisms do not grow well and do not reproduce on rich agars. Yet, with the exception of *P. infestans* and *P. phaseoli*, all made a vigorous growth on a rich medium, and a number of them formed sporangia.

While table 3 crystallizes certain definite reactions, these are, nevertheless, still of secondary importance, and should never be used as the principal step towards the segregation of groups of species. Normal fluctuations, certain peculiarities of saltants, or the less fixed characteristics of widely separated strains are more likely to manifest themselves on solid agars than in liquid cultures. For this reason it is best to crowd the solid-agar work as much into the background as is feasible.

Table 4 contains results which are of much greater significance. Unfortunately, only a few fungi are involved, as a great number failed to produce oögonia on malt-extract and nucleinic-acid agars. It has been stated that the presence or absence of oögonia is not of primary specific value; but when an organism forms oögonia in one nutrient medium and fails to do so in another, then the appearance of the sexual phase assumes a greater significance because a possible heterothallism can no longer be considered a factor. It seems, however, that the quantity of oögonia on solid agars greatly varies even when all environmental conditions remain the same. *P. capsici*, for example, has at times formed a fair quantity of oögonia on malt-extract agar; but this occurred when it was freshly isolated from its host. After being carried in pure culture for two years the number of oögonia which may form on this agar has been reduced to a minimum. Similarly, the oögonia of *P. cactorum* have varied from II to IV in quantity, and a decrease of oögonia has usually been accompanied by an increase of sporangia or *vice versa*. When *P. cactorum* 3 was first isolated from the apple fruit, it formed such an enormous number of oögonia on malt-extract agar that the field of the microscope presented a solid mass of oögonia. After two years on artificial media the quantity of oögonia has been reduced to such an extent that sometimes this fungus gives rise to no oögonia at all, although subsequent trials may reward the worker with a fair number (III) of these bodies. No. 139 at first formed a few oögonia on malt-extract agar, but several months later, when new trials were made, no oögonia could be found. Very recently, a strain of *P. parasitica* isolated from Bryophyllum in Bermuda by A. Berg and identified by the writer was found to form a few oögonia in malt-extract agar; it is very probable that no such bodies will be produced after the fungus is carried on artificial media for a few months.

The ability of some Phytophthora species to produce oögonia is retained much longer on oatmeal agar; eventually, however, these sexual bodies appear less frequently and in fewer numbers, while the chlamydospores become more and more abundant. It is not improbable that after a long period of culture upon artificial media these organisms will lose their ability to form oögonia.

There seems to be a cycle or periodicity in the life of these and similar organisms. Not only oögonial production but the development of sporangia, of aërial hyphae, of the type of colonies, and even the ability to make a growth on certain agars, seem to be influenced by this cycle. When attempts were made to grow *P. colocasiae* on the concentrated malt-extract agar, for example, three successive attempts gave negative results; a fourth attempt was successful, and a fairly good colony was the result. *P. mexicana* and *P. nicotianae* were attempted three and four times respectively on a more concentrated malt-extract agar (thirty times the normal concentration) before positive results were obtained. This becomes all the more remarkable when it is remembered that all cultures were made from the same tube, that the inoculum in all cases consisted of a generous portion of the medium and the mycelium, and that all environmental conditions were the same. *P. infestans* has also manifested some remarkable cases of periodicity. For nearly a year it grew remarkably well in the nutrient solution and formed large colonies within four or five days after the transfer of the inoculum into the solution. Then, suddenly, a changed behavior was seen; the inoculum produced very little or no growth in the nutrient solution even a month after the transfer. This was repeated time and again, but the results remained the same. Similar examples will be given throughout this work to illustrate that there is a definite periodicity in the life cycle of these fungi.

*P. erythroseptica* forms oögonia (III) when grown on the following agars:

*Calcium-nitrate Agar*

Dihydrogen potassium phosphate.....	0.65 g.
Magnesium sulfate.....	0.3 g.
Calcium nitrate.....	1.7 g.
Dextrose.....	3.6 g.
Bacto agar.....	20 g
Distilled water.....	1000 cc.

*Glutamic-acid Agar*

Dihydrogen potassium phosphate.....	0.65 g.
Magnesium sulfate.....	0.3 g.
Glutamic acid.....	0.7 g.
Dextrose.....	3.6 g.
Bacto agar.....	25 g.
Distilled water.....	1000 cc.

*P. capsici* and *P. phaseoli* produced a great number (IV and V respectively) of oögonia on oatmeal agar, while *P. cryptogea* formed a fair quantity (II) of these bodies. A number of other species also gave rise to oögonia on this medium, but they have been omitted from this list because in their case the formation of sexual bodies was found to have no bearing upon their final classification.

Since *P. cactorum*, *P. fagi*, *P. capsici*, *P. erythroseptica*, and no. 372

produce oögonia on malt-extract, nucleinic-acid, calcium-nitrate and glutamic-acid agars, they can at once be separated from the rest. *P. capsici*, however, may better be omitted because of the limited number of its oögonia.

*P. infestans* and *P. phaseoli* can easily be separated when grown in test tubes of oatmeal agar. *P. infestans* is so uncertain in the formation of sexual bodies, and these, when formed, are so unusually rare that any one would be justified in classifying it as giving negative results in this respect. *P. phaseoli*, on the other hand, produces an unusually large number (IV) of oögonia.

Summing up the results obtained with solid media, we find the following starting-points: plate cultures of malt-extract and nucleinic-acid media segregate *P. infestans* and *P. phaseoli*, while oatmeal agar separates these two from each other. However, if no other distinguishing factors were available, this separation would not be absolutely safe. We shall later see that some saltants may drop oögonia from their life cycle or may reduce the number of these bodies to a minimum.

### Liquid Cultures

*The effect of presence and absence of food.* The influence of food upon the growth and reproduction of fungi has received considerable attention. In view of the fact that the writer only recently (3) summarized the literature concerning this type of work, no references will be given here. The results given in table 5 seem to contradict the generalization of Klebs and others concerning the unfavorable influence of food, especially rich food, upon the reproduction of fungi, notably Phycomycetes.

Five experiments were made to determine the effect of food upon the reproduction of Phytophthoras:

1. The organisms were grown in 5 cc. of the nutrient solution, and two weeks later were examined for fruiting.
2. The young colonies were transferred daily to a fresh supply (5 cc.) of the nutrient solution. This daily transference was continued for two weeks. The object was to keep the mycelium in vigorously growing condition and to eliminate any possible staling effect.
3. Vigorously growing and three-day-old colonies were washed in distilled water and were then transferred to 2 cc. of fresh nutrient solution. Three days later they were examined for sporangia.
4. Same as no. 3, except that the colonies were transferred to concentrated nutrient solution (ten times the usual concentration).
5. Same as no. 3, except that the colonies were transferred to 2 cc. of distilled sterile water.

Table 5 gives the results.

Some significant data are to be found in table 5. In the first place, *P. cinnamomi* is at once distinguished because it is the only organism that

forms chlamydospores but no sporangia or oögonia. No. 372 can be identified still more easily because it is the only organism which produces oögonia, whereas the others either remain sterile or give rise to sporangia or chlamydospores. *P. capsici*, *P. citrophthora*, *P. cryptogea*, *P. erythro-septica*, *P. mexicana*, nos. 8, 26, 36, 44, 121, and 130 are segregated into a group which remains sterile in the presence of food. However, *P. erythro-septica*, *P. mexicana*, nos. 8 and 130 remain sterile even when food is withdrawn. Perhaps *P. cryptogea* should also be included here because of its very scanty and rare production of sporange-like bodies in distilled water. *P. erythro-septica* can be separated readily from this group, because, as we have seen, it forms oögonia on solid agars. *P. cactorum* strains and *P. fagi* still remain as a group, and so do *P. infestans* and *P. phaseoli*. *P. arecae* and *P. nicotianae* do not reproduce readily in the presence of food, but *P. nicotianae* gives rise to a great number of sporangia in distilled water, while *P. arecae* yields only a few.

*P. parasitica-rhei* IV shows a rather remarkable phenomenon: if grown

TABLE 5. *The Effect of Presence and Absence of Food on Sporangium-production*

Organisms	Grown in 5 cc. Solution (14 Days at 20° C.)	Transferred Daily (14 Days at 20° C.)	Transferred to 2 cc. of Solution (3 Days at 20° C.)	Transferred to Concentrated Solution (3 Days at 20° C.)	Transferred to Distilled Water (3 Days at 20° C.)
<i>P. arecae</i> .....	II	II	I	I	I
<i>P. cactorum</i> 1.....	IV	III	III	III	III
<i>P. cactorum</i> 2.....	IV	III	III	III	III
<i>P. cactorum</i> 3.....	IV	III	III	II	III
<i>P. fagi</i> .....	IV	IV	III	III	IV
<i>P. capsici</i> .....	o	o	o	o	III
<i>P. citrophthora</i> .....	I	o	I	o	III
<i>P. cinnamoni</i> .....	o	o	o	o	o
<i>P. cryptogea</i> .....	o	o	o	o	I
<i>P. erythro-septica</i> .....	o	o	o	o	o
<i>P. mexicana</i> .....	o	o	o	o	o
<i>P. infestans</i> .....	III	III	III	III	III
<i>P. phaseoli</i> .....	III	III	III	IV	III
<i>P. nicotianae</i> .....	II	I	o	o	IV
<i>P. syringae</i> .....	IV	IV	III	II	III
<i>P. parasitica</i> .....	IV	IV	III	III	IV
<i>P. terrestris</i> .....	III	II	III	II	IV
<i>P. sp.</i> (Reddick's) I...	IV	II	III	III	IV
<i>P. sp.</i> (Reddick's) II...	IV	IV	III	III	IV
<i>P. parasitica-rhei</i> I...	IV	III	III	II	IV
<i>P. parasitica-rhei</i> II...	II	II	III	II	III
<i>P. parasitica-rhei</i> III...	II	II	III	I	IV
<i>P. parasitica-rhei</i> IV...	III	o	II	I	III

TABLE 5 (Continued)

Organisms	Grown in 5 cc. Solution (14 Days at 20° C.)	Transferred Daily (14 Days at 20° C.)	Transferred to 2 cc. of Solution (3 Days at 20° C.)	Transferred to Concentrated Solution (3 Days at 20° C.)	Transferred to Distilled Water (3 Days at 20° C.)
<i>P. parasitica-rhei</i> V...	IV	IV	III	II	IV
<i>P. faberi</i> .....	IV	IV	III	IV	III
<i>P. palmivora</i> .....	IV	IV	II	IV	III
<i>P. colocasiae</i> .....	III	II	III	II	IV
139.....	III	III	III	III	III
142.....	IV	IV	IV	III	IV
143.....	III	II	III	II	II
145.....	IV	III	IV	II	III
123.....	IV	IV	IV	IV	III
126.....	III	III	III	II	IV
136.....	IV	IV	III	III	III
127.....	IV	IV	IV	III	IV
137.....	III	III	III	III	III
138.....	IV	III	IV	III	IV
140.....	III	III	IV	III	IV
144.....	III	II	IV	II	III
22.....	o-II	o	o-III	o	III
26.....	o	o	o	o	III
97.....	o-III	o	o-II	o	III
100.....	o	o	o-II	o	II
102.....	o-I	o	o-II	o	II
117.....	o-I	o	o-II	o	III
116.....	o	o	o-I	o	III
141.....	o-II	o	o-II	o-II	III
8.....	o	o	o	o	o
36.....	o	o	o	o	III
44.....	o	o	o	o	III
121.....	o	o	o	o	I
130.....	o	o	o	o	o
372.....	Spor. Oög. o III	Spor. Oög. o II	Spor. Oög. o III	Spor. Oög. o IV	Spor. Oög. II II

undisturbed in 5 cc. of nutrient solution, this strain produces sporangia without any difficulty; however, if it is transferred to a fresh supply of this solution once a day for two weeks or longer, it remains sterile; soon after the daily transfers are discontinued, however, the production of sporangia begins. No adequate explanation can be offered. It is probable that the fungus requires free access to atmospheric oxygen, and the successive transfers prevent the mycelium from growing toward the surface of the solution. Also, the mechanical disturbances caused by the transferring operations clump the hyphal threads together, and before they have had much chance to grow to the surface of the solution the subsequent transfer repeats the process. It should be remembered, in this connection, that *P. parasitica-rhei* IV not only comes from, but it may also give rise to, *P. parasitica-rhei* I; this latter organism manifests quite different reactions from those shown by its filial strain. With the exception of the negative result obtained with *P. parasitica-rhei* IV (table 5, columns 2 and 5), all the organisms from *P. parasitica* down to no. 144 inclusive show a definite

uniformity of reactions. Nos. 22, 97, 100, 102, 117, 116, and 141 exhibit, once more, the interesting phenomenon of physiological saltation, sterile forms yielding fertile strains, and fertile organisms producing sterile strains. In this connection we should bear in mind that all the colonies involved in these saltations came from the same cultures, that all the solutions were of the same quality and quantity, and all environmental conditions were identical, yet at one time the colony from a given culture gave a negative reaction while a sister colony yielded a positive reaction. These experiments have been repeated often enough to convince the writer that this is a well established phenomenon worthy of close attention.

In most cases the presence of food is just as conducive to sporangium formation as the absence of food. However, *P. capsici*, *P. citrophthora*, *P. nicotianae*, nos. 26, 36, and 44 give rise to very few or no sporangia in the presence of food, while in distilled water a more free reproduction is to be found.

While, generally speaking, reproduction is not very distinctly better when the colonies remain undisturbed in 5 cc. of nutrient solution than when they are transferred daily to a fresh supply, in no case is a daily transfer to fresh nutrient solution conducive to better reproduction. Since growth always precedes reproduction, it follows that when a fresh food is supplied to a given organism there will be more growth than reproduction. The staling effect of an organism upon a solution in which it grows may eventually hinder growth and promote reproduction. This was demonstrated experimentally. *P. cactorum*, *P. syringae*, *P. capsici*, *P. faberi*, and *P. cryptogea* were grown for two months in 250 cc. of the nutrient solution in flasks of 500 cc. capacity; then the solution was filtered aseptically and two cc. of it was placed in each of a series of culture dishes. Fresh nutrient solution was used as a check. Vigorously growing colonies of the organisms were washed in sterile distilled water and transferred to these dishes. Three days later the cultures were examined for new growth and reproduction. In all cases the checks grew very vigorously while the cultures in the staled solutions grew very little or not at all. No such sharp difference was noticed, however, in the reproductive processes. Cultures in staled solutions reproduced as well as those in the checks. However, the solution staled by *P. cryptogea* induced a better reproduction than the checks.

✓ The staling phenomenon has received much attention lately, especially in England, and cases have been reported in which toxic substances given off by the growing colony have diffused into the agar and prevented further growth. No such phenomenon has been observed in *Phytophthoras*, and the colonies have grown without any apparent diminishing rate, as can be seen in the following experiment. Twenty-five cc. of malt-extract agar was poured in a carius tube (70 cm. long with 60 cm. to the constriction) and sterilized. This afforded a flat growing surface 60 cm. long. Transfers

were made to these tubes, and the daily growth was measured for forty days. The rate of growth remained constant throughout this period, thus demonstrating that the diffusion into agar of growth-inhibiting substances was not of sufficient consequence to become a factor.

In the dishes where the cultures were undisturbed, however, the colony was able to exert some staling influence upon the solution in which it was growing, and subsequently upon its own sporulation. This perhaps explains the numerous negative results shown in the second column of table 5. However, it does not explain the fact that a large number of organisms continued to reproduce in spite of the daily renewal of the food. Perhaps if the nature of the food were changed and the transfers were made more frequently, the results would be considerably different.

Most of the organisms were able to reproduce in the concentrated solution. All the colonies of these strains, after being transferred to this rich food, and even to the less concentrated solution (table 5, column 3), kept on growing vigorously; nevertheless, sporangia were formed. This fact demonstrates that growth and reproduction may go on at the same time and in the same colony, and that portions of even a coenocytic hypha may

TABLE 6. *The Effect of M/100 Solutions of Monosaccharides on Sporangium-production*

Organisms	Dextrose	Levulose	Mannose	Galactose	Xylose
<i>P. arecae</i> .....	II	II	I	II	III
<i>P. cactorum</i> 1.....	III	IV	IV	III	IV
<i>P. cactorum</i> 2.....	III	IV	III	IV	III
<i>P. cactorum</i> 3.....	III	IV	IV	IV	III
<i>P. fagi</i> .....	III	III	IV	IV	III
<i>P. capsici</i> .....	III	III	III	III	I
<i>P. citrophthora</i> .....	III	II	III	III	III
<i>P. cinnamoni</i> .....	o	o	o	o	o
<i>P. cryptogea</i> .....	o	o	o	o	o
<i>P. erythrosepica</i> .....	o	o	o	o	o
<i>P. mexicana</i> .....	o	o	o	o	o
<i>P. infestans</i> .....	III	III	IV	IV	IV
<i>P. phaseoli</i> .....	III	III	III	IV	III
<i>P. nicotianae</i> .....	o	I	o	III	III
<i>P. syringae</i> .....	III	III	IV	IV	IV
<i>P. parasitica</i> .....	IV	IV	IV	V	IV
<i>P. terrestris</i> .....	IV	III	III	IV	IV
<i>P. sp.</i> (Reddick's) I...	IV	III	III	V	IV
<i>P. sp.</i> (Reddick's) II...	III	IV	IV	IV	IV
<i>P. parasitica-rhei</i> I...	IV	III	III	V	IV
<i>P. parasitica-rhei</i> II...	III	III	III	III	III
<i>P. parasitica-rhei</i> III...	III	III	III	IV	IV
<i>P. parasitica-rhei</i> IV...	o-II	I-III	o-III	III	II

TABLE 6 (Continued)

Organisms	Dextrose	Levulose	Mannose	Galactose	Xylose
<i>P. parasitica-rhei</i> V...	IV	IV	IV	IV	IV
<i>P. faberi</i> .....	III	III	III	IV	IV
<i>P. palmivora</i> .....	III	III	III	IV	III
<i>P. colocasiae</i> .....	III	III	II	II	II
139.....	III	IV	III	IV	III
142.....	III	IV	IV	IV	III
143.....	III	IV	III	IV	III
145.....	IV	IV	III	IV	IV
123.....	III	III	IV	IV	IV
126.....	III	III	IV	IV	IV
136.....	III	III	III	III	III
127.....	IV	III	IV	IV	IV
137.....	IV	III	IV	IV	III
138.....	IV	III	IV	V	IV
140.....	IV	IV	III	IV	IV
144.....	III	IV	III	IV	IV
22.....	III	III	III	III	III
26.....	II	I	I	I	II
97.....	II	II	II	II	II
100.....	II	o-II	o-II	II	II
102.....	II	II	II	II	III
117.....	II	II	II	II	II
116.....	o-III	o-III	o-II	II	II
141.....	III	III	III	II	III
8.....	o	o	o	o	o
36.....	o-II	o-II	o-II	o-II	o-II
44.....	o	o-II	o-III	o-II	o-III
121.....	o	o	o	o	o
130.....	o-I	o-II	o-III	o-III	o-III
372.....	Spor. Oög. II III	Spor. Oög. II IV	Spor. Oög. II III	Spor. Oög. II III	Spor. Oög. II III

be in different physiological conditions so that while one portion is growing vigorously another portion may be actively reproducing in spite of its contact with a fresh nutrient solution. *P. parasitica-rhei* II and III reproduced very scantily in the concentrated solution, and, together with *P. parasitica-rhei* IV, formed the only sharp deviations from the uniform reactions of the organisms listed from *P. parasitica* down to and including no. 144.

No. 372 stands by itself. The readiness with which it forms oögonia is most remarkable. The presence of food even in high concentration encourages the formation of oögonia and suppresses that of sporangia, while in distilled water oögonia and sporangia are produced in equal abundance.

### The Effect of Sugars

M/100 solutions of nine different sugars were prepared, and the colonies were transferred to 2 cc. of each solution. Unless otherwise stated, all the various solutions mentioned hereafter are of the same concentration and quantity, and all cultures were examined three days after the transfer.



At first another concentration (M/10) was used, but it was abandoned because in its effect upon reproduction it was not definitely different from the more dilute solution.

Tables 6 and 7 show that the following groups, which began to be apparent in the previous experiments, still remain intact: group 1, *P. cactorum* strains and *P. fagi*; group 2, *P. erythroseptica* and *P. mexicana*; group 3, *P. infestans* and *P. phaseoli*; group 4, consisting of twenty-four organisms from *P. parasitica* to no. 144 inclusive. The peculiar action of *P. parasitica-rhei* IV suggests the possibility that group 5, composed of nos. 22, 26, 97, 100, 102, 117, 116, and 141, may be connected with group 4. The sixth and last group consists of nos. 8, 36, 44, 121, and 130. Nos. 8 and 121 have been so consistently sterile that they may at once be recognized and separated from other members of this group. *P. arecae* shows no relationship to any group; nor does *P. nicotianae*. *P. capsici* and *P. citrophthora* manifest definite similarities, but they can be separated by the fact that the former produces oögonia in oatmeal agar in great abundance while the latter does not; furthermore, *P. capsici* forms only a few sporangia

TABLE 7. *The Effect of M/100 Solutions of Di- and Trisaccharides on Sporangium-production*

Organisms	Sucrose	Maltose	Lactose	Raffinose
<i>P. arecae</i> .....	I	II	I	II
<i>P. cactorum</i> 1.....	IV	III	III	III
<i>P. cactorum</i> 2.....	IV	III	III	IV
<i>P. cactorum</i> 3.....	IV	III	III	IV
<i>P. fagi</i> .....	IV	IV	IV	III
<i>P. capsici</i> .....	III	III	IV	III
<i>P. citrophthora</i> .....	II	III	I	III
<i>P. cinnamomi</i> .....	o	o	o	o
<i>P. cryptogea</i> .....	o	o	o	o
<i>P. erythroseptica</i> .....	o	o	o	o
<i>P. mexicana</i> .....	o	o	o	o
<i>P. infestans</i> .....	III	III	III	IV
<i>P. phaseoli</i> .....	III	III	III	IV
<i>P. nicotianae</i> .....	I	I	II	III
<i>P. syringae</i> .....	IV	III	III	IV
<i>P. parasitica</i> .....	III	IV	IV	V
<i>P. terrestris</i> .....	III	IV	IV	IV
<i>P. sp.</i> (Reddick's) I.....	IV	IV	IV	IV
<i>P. sp.</i> (Reddick's) II.....	III	III	IV	IV
<i>P. parasitica-rhei</i> I.....	III	IV	IV	IV
<i>P. parasitica-rhei</i> II.....	III	III	III	IV
<i>P. parasitica-rhei</i> III.....	III	IV	IV	IV
<i>P. parasitica-rhei</i> IV.....	o-II	o-II	I-II	I-III

TABLE 7 (Continued)

Organisms	Sucrose	Maltose	Lactose	Raffinose
<i>P. parasitica-rhei</i> V. ....	III	III	IV	IV
<i>P. faberi</i> .....	III	III	III	IV
<i>P. palmivora</i> .....	II	III	III	IV
<i>P. colacasiae</i> .....	II	II	II	II
139. ....	III	III	III	IV
142. ....	III	IV	IV	IV
143. ....	III	III	III	IV
145. ....	III	IV	III	IV
123. ....	III	III	IV	IV
126. ....	III	III	III	III
136. ....	III	III	III	III
127. ....	IV	IV	IV	IV
137. ....	III	IV	IV	IV
138. ....	IV	IV	IV	IV
140. ....	IV	IV	III	IV
144. ....	III	IV	III	IV
22. ....	III	III	III	III
26. ....	II	II	II	III
97. ....	II	II	III	II
100. ....	II	II	o-II	III
102. ....	II	II	II	II
117. ....	II	II	III	III
116. ....	II	II	II	o-II
141. ....	II	III	III	IV
8. ....	o	o	o-I	o
36. ....	o-II	o-II	o-II	o-II
44. ....	o-I	o-II	o-III	o-III
121. ....	o	o	o	o
130. ....	o-III	o-III	o-III	o-III
372. ....	Spor. Oög. I III	Spor. Oög. I III	Spor. Oög. II III	Spor. Oög. II III

in xylose while *P. citrophthora* yields a good quantity, the reverse being the case when lactose is substituted for xylose. *P. cryptogea* develops no sporangia in agars, nor in any of the nutrient solutions or any of the sugars. In this respect it occupies a unique position among Phytophthoras.

It is remarkable that there are so small differences in the effects of the various sugars. Generally speaking, however, raffinose induces the most abundant reproduction.

Not very many striking reactions can be found in table 8. While group I still remains intact, *P. erythro-septica* and *P. mexicana* split apart in their reaction toward glycine and alanine. *P. cryptogea*, *P. erythro-septica*, and no 121 remain sterile in all four solutions. Nos. 22, 26, and 97 yield no sporangia in leucine, while nos. 100, 8, 36, 44, and 130 remain sterile in both leucine and isoleucine. *P. parasitica-rhei* IV, nos. 116 and 44 saltate in some of the solutions. In view of the fact that the last two groups are not at all stable in their reactions, it is highly probable that more of them would show saltations if the experiments were repeated a larger number of times. No. 372, which until this experiment had produced

oögonia in abundance, yielded only a very few of these bodies in alanine and none in the other solutions. It gave rise to sporangia in glycine and alanine only. Apparently isoleucine suppresses the formation of sporangia in *P. arecae*, although no such effect is to be noticed in a large number of other organisms.

Aspartic and glutamic acids prevent reproduction in the first fifteen organisms listed in table 9, as well as in nos. 8, 36, 44, 121, 130, and 372. Most of the members of groups 4 and 5, on the other hand, saltate back and forth in these amino acids and show the close relationship and the physiological unity of many of their members. Although *P. parasitica-rhei* III, *P. faberi*, *P. colocasiae*, nos. 139, 142, 123, 138, 26, 97, and 100 yield only positive reactions, we should not be led to conclude that these organisms are definitely fixed in their reactions towards aspartic and glutamic acids. It is probable that if the experiments were repeated often enough some negative results would also be obtained.

An effort was made to segregate the positive and the negative phases from *P. parasitica*, *P. terrestris*, *P. parasitica-rhei* I, and Reddick's organism.

TABLE 8. *The Effect of M/100 Solutions of Monoamino-monocarboxylic Acids on Sporangium-formation*

Organisms	Glycine	Alanine	Leucine	Isoleucine
<i>P. arecae</i> .....	IV	III	II	I
<i>P. cactorum</i> 1.....	III	III	III	IV
<i>P. cactorum</i> 2.....	III	IV	IV	IV
<i>P. cactorum</i> 3.....	III	IV	IV	III
<i>P. fagi</i> .....	III	III	III	III
<i>P. capsici</i> .....	III	IV	II	II
<i>P. citrophthora</i> .....	III	III	I	o
<i>P. cinnamoni</i> .....	o	o	o	o
<i>P. cryptogea</i> .....	o	o	o	o
<i>P. erythrosepica</i> .....	o	o	o	o
<i>P. mexicana</i> .....	II	II	o	o
<i>P. infestans</i> .....	IV	III	III	III
<i>P. phaseoli</i> .....	III	III	III	III
<i>P. nicotianae</i> .....	II	III	III	III
<i>P. syringae</i> .....	I	III	IV	IV
<i>P. parasitica</i> .....	V	IV	IV	IV
<i>P. terrestris</i> .....	IV	IV	IV	IV
<i>P. sp.</i> (Reddick's) I.....	V	IV	IV	IV
<i>P. sp.</i> (Reddick's) II.....	IV	IV	III	II
<i>P. parasitica-rhei</i> I.....	IV	IV	III	IV
<i>P. parasitica-rhei</i> II.....	IV	IV	II	II
<i>P. parasitica-rhei</i> III.....	IV	IV	III	III
<i>P. parasitica-rhei</i> IV.....	I-IV	III	I-III	II

TABLE 8 (Continued)

Organisms	Glycine	Alanine	Leucine	Isoleucine
<i>P. parasitica-rhei</i> V. ....	IV	IV	II	II
<i>P. faberi</i> .....	III	IV	II	III
<i>P. palmivora</i> .....	II	IV	II	II
<i>P. colocasiae</i> .....	III	II	III	III
130.....	IV	III	III	IV
142.....	V	V	III	IV
143.....	III	IV	II	II
145.....	IV	IV	IV	IV
123.....	IV	IV	III	III
126.....	III	III	IV	IV
136.....	III	IV	III	III
127.....	IV	V	III	III
137.....	III	III	II	IV
138.....	IV	IV	IV	IV
140.....	III	IV	III	IV
144.....	III	IV	III	IV
22.....	IV	IV	o	III
26.....	II	II	o	II
97.....	III	III	o	I
100.....	II	II	o	o
102.....	III	III	o	III
117.....	III	II	o	II
116.....	III	IV	o-IV	III
141.....	IV	IV	o	o
8.....	II	II	o	o
36.....	III	II	o-I	o-II
44.....	III	II	o	o
121.....	o	o	o	o
130.....	II	II	o	o
372.....	Spor. Oög. III o	Spor. Oög. II I	Spor. Oög. o o	Spor. Oög. o o

Selections were made for several generations; when a colony failed to form any sporangia in aspartic acid, transfers were made from it to the nutrient solution and the ensuing colonies were again transferred to aspartic acid; the same was done with colonies which gave positive reactions. But when the mycelium was examined for sporangia, it was found that a negative colony would give rise to a positive colony and a positive colony would produce a negative colony. Or perhaps a colony would breed true for a generation or two, and then would revert to its old habits. This type of phenomenon is well illustrated in the chart given a little later.

The behavior of groups 4 and 5 towards aspartic and glutamic acids at once distinguishes them from all other *Phytophthora* species.

*P. infestans* and *P. phaseoli* split apart in their reaction toward arginine. Nos. 372, 8, and 121, *P. nicotianae*, *P. syringae*, *P. infestans*, *P. mexicana*, *P. erythroseptica*, *P. cryptogea*, and *P. cinnamoni* fail to reproduce in arginine, while nos. 22, 26, 97, 100, 102, 117, 116, 141, 36, 44, and 130 register a negative result in this amino acid at one time, and a positive result at another time.

Ammonium nitrate seems to be the least favorable of the four nitrates whose effects are summarized in table 10. Fourteen organisms registered a negative reaction in this solution and six others gave a very slight positive reaction. On the other hand, magnesium, potassium, and calcium nitrates each yielded only four negative reactions.

Nos. 8, 36, 44, 121, and 130 still manifest a physiological saltation, although all others seem to be more stable in their reproduction.

Generally speaking, nitrates stimulate sporangial reproduction. While no. 372 finds ammonium nitrate too toxic, it reproduces well, both sexually and asexually, in the remaining three nitrates.

Groups 4 and 5 are still seen to remain together. This serves to strengthen our belief that the two have too many characteristics in common to contain more than one, or possibly two, distinct species. The saltants of Reddick's *Phytophthora* and of *P. parasitica-rhei* have quite constantly exhibited a uniformity of behavior, which fact lends weight to the argument that such characteristics as they have manifested on solid agars can not be

TABLE 9. *The Effect of M/100 Solutions of Monoamino-monocarboxylic Acids, and of a Diamino-Monocarboxylic Acid on Sporangium-formation*

Organisms	Aspartic Acid	Glutamic Acid	Arginine
<i>P. arecae</i> .....	o	o	I
<i>P. cactorum</i> 1.....	o	o	IV
<i>P. cactorum</i> 2.....	o	o	IV
<i>P. cactorum</i> 3.....	o	o	IV
<i>P. fagi</i> .....	o	o	II
<i>P. capsici</i> .....	o	o	III
<i>P. citrophthora</i> .....	o	o	III
<i>P. cinnamomi</i> .....	o	o	o
<i>P. cryptogea</i> .....	o	o	o
<i>P. erythroseptica</i> .....	o	o	o
<i>P. mexicana</i> .....	o	o	o
<i>P. infestans</i> .....	o	o	o
<i>P. phaseoli</i> .....	o	o	III
<i>P. nicotianae</i> .....	o	o	o
<i>P. syringae</i> .....	o	o	o
<i>P. parasitica</i> .....	o-IV	o-IV	IV
<i>P. terrestris</i> .....	o-IV	o-IV	IV
<i>P. sp.</i> (Reddick's) I.....	o-III	o-III	IV
<i>P. sp.</i> (Reddick's) II.....	o-IV	o-IV	III
<i>P. parasitica-rhei</i> I.....	o-III	o-III	IV
<i>P. parasitica-rhei</i> II.....	o-IV	o-IV	IV
<i>P. parasitica-rhei</i> III.....	III	IV	IV
<i>P. parasitica-rhei</i> IV.....	o-II	o-II	III

TABLE 9 (Continued)

Organisms	Aspartic Acid		Glutamic Acid		Arginine	
<i>P. parasitica-rhei</i> V. ....	o-IV		o-III		IV	
<i>P. faberi</i> .....	III		III		III	
<i>P. palmivora</i> .....	o-III		o-III		II	
<i>P. colcasiae</i> .....	II		II		II	
139.....	III		III		IV	
142.....	II		II		IV	
143.....	o-III		o-III		III	
145.....	o-II		o-II		III	
123.....	III		III		III	
126.....	o-III		o-III		III	
136.....	o-II		o-IV		III	
127.....	o-IV		o-III		IV	
137.....	o-III		o-II		IV	
138.....	III		III		III	
140.....	o-III		o-IV		II	
144.....	o-IV		o-III		IV	
22.....	o-III		o-II		o-II	
26.....	II		II		o-II	
97.....	III		III		o-I	
100.....	I		II		o-II	
102.....	o-II		o-II		o-II	
117.....	o-II		o-II		o-II	
116.....	o-II		o-II		o-I	
141.....	o-I		o-II		o-IV	
8.....	o		o		o	
36.....	o		o		o-II	
44.....	o		o		o-I	
121.....	o		o		o	
130.....	o		o		o-II	
372.....	Spor.	Oög.	Spor.	Oög.	Spor.	Oög.
	o	o	o	o	o	o

of any specific value unless substantiated by a distinct dissimilarity of other physiological activities.

We see in table 11 that, while the *P. cactorum* strains find sodium phosphate uniformly unfavorable for reproduction, *P. fagi* remains unaffected by this solution. *P. infestans* produces sporangia very sparingly in all three of the phosphates, while *P. phaseoli* does a little better. Group 4 is quite uniform in its favorable reaction towards the phosphates, while groups 5 and 6 still saltate back and forth. Ammonium phosphate, in spite of the toxic property of ammonium, becomes a good agent for the production of sporangia. Phosphorus, being a strong neutralizing substance, serves to keep the toxic property of ammonium in check.

If we now turn to table 12, we find that ammonium sulfate is decidedly more unfavorable than either potassium or magnesium sulfate; exclusive of the four organisms which have, in most cases, remained sterile, there are fifteen which fail to give rise to any sporangia in ammonium-sulfate solution, while eight register a very weak positive reaction. Potassium and magnesium sulfates, on the other hand, check or suppress reproduction in only a few organisms.

Ammonium chlorid is the least favorable of the three salts; seventeen organisms failed to produce sporangia in M/100 solution of this substance as against five in sodium and in magnesium chlorid. The toxicity of ammonium salts has been recorded by other investigators, and no further comment need be made here.

The various groups which were formed in previous experiments still retain their group solidarity in the chlorids. When we pass on to the carbonate, however, we find that *P. mexicana* dissociates itself from *P. erythroseptica*, and *P. infestans* from *P. phaseoli*.

A number of the organisms produced dwarfed sporangia in potassium carbonate, as well as in some of the ammonium salts mentioned in the previous tables, and in a 15-percent glycerin. Text figure 5 illustrates this very well.

### THE SALTATION PHENOMENON

Before a final analysis of the experimental data is made, it is necessary, for a clearer interpretation of the results, to study the saltation phenomenon in somewhat greater detail.

TABLE 10. *The Effect of M/100 Solutions of Nitrates on Sporangium-formation*

Organisms	Ammonium Nitrate	Magnesium Nitrate	Calcium Nitrate	Potassium Nitrate
<i>P. arecae</i> .....	I	II	III	II
<i>P. cactorum</i> 1.....	II	IV	IV	IV
<i>P. cactorum</i> 2.....	III	IV	V	IV
<i>P. cactorum</i> 3.....	IV	IV	V	IV
<i>P. fagi</i> .....	III	IV	IV	IV
<i>P. capsici</i> .....	II	III	IV	IV
<i>P. citrophthora</i> .....	I	IV	IV	IV
<i>P. cinnamomi</i> .....	o	o	o	o
<i>P. cryptogea</i> .....	o	o	o	o
<i>P. erythroseptica</i> .....	o	II	II	II
<i>P. mexicana</i> .....	o	II	III	III
<i>P. infestans</i> .....	I	III	III	I
<i>P. phaseoli</i> .....	II	II	II	III
<i>P. nicotianae</i> .....	IV	IV	IV	V
<i>P. syringae</i> .....	o	III	II	II
<i>P. parasitica</i> .....	III	V	IV	V
<i>P. terrestris</i> .....	IV	IV	IV	III
<i>P. sp.</i> (Reddick's) I.....	III	IV	IV	IV
<i>P. sp.</i> (Reddick's) II.....	IV	IV	V	IV
<i>P. parasitica-rhei</i> I.....	III	IV	IV	IV
<i>P. parasitica-rhei</i> II.....	III	IV	IV	IV
<i>P. parasitica-rhei</i> III.....	III	IV	IV	IV
<i>P. parasitica-rhei</i> IV.....	II	III	III	III

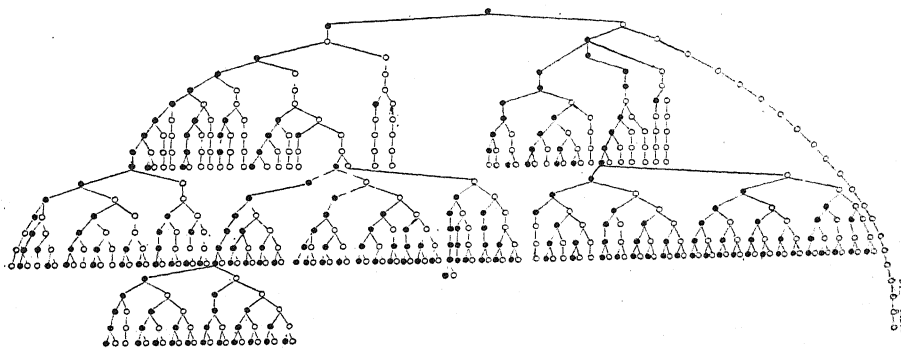
TABLE IO (Continued)

Organisms	Ammonium Nitrate	Magnesium Nitrate	Calcium Nitrate	Potassium Nitrate
<i>P. parasitica-rhei</i> V.....	III	IV	IV	IV
<i>P. faberi</i> .....	IV	IV	IV	IV
<i>P. palmivora</i> .....	III	IV	IV	IV
<i>P. colocasiae</i> .....	II	III	III	III
I39.....	III	V	V	V
I42.....	III	V	V	V
I43.....	II	IV	V	V
I45.....	II	IV	IV	IV
I23.....	IV	IV	V	V
I26.....	III	V	V	V
I36.....	III	IV	V	IV
I27.....	IV	IV	IV	IV
I37.....	III	IV	V	IV
I38.....	III	IV	IV	IV
I40.....	III	IV	IV	IV
I44.....	III	IV	IV	IV
22.....	I	IV	IV	IV
26.....	o	III	III	III
97.....	o	III	III	III
100.....	o	III	III	III
102.....	I	III	III	III
117.....	I	IV	III	III
116.....	II	II	II	II
141.....	II	III	IV	III
8.....	o	o-II	o-I	o-II
36.....	o	o	o-I	o-II
44.....	o	o-II	o-I	o-III
I21.....	o	o	o	o-I
I30.....	o	o-II	o	o-II
372.....	Spor. Oög. o o	Spor. Oög. II IV	Spor. Oög. III IV	Spor. Oög. III IV

*P. parasitica-rhei* I is most interesting in this respect. For nearly seven months after this organism was received, it behaved normally. One day, however, a colony in a malt-extract-agar plate gave rise to an entirely distinct form (Pl. XLIX, fig. 1). At first it was believed to be a contamination, but, when it recurred regularly, this notion was abandoned. Whenever a transfer was made from a tube of *P. parasitica-rhei* to a malt-extract-agar plate, the ensuing colony almost invariably split into the typical growth and the new form. Transfers made from these two sectors gave rise to two distinct colonies, as can be seen in Plate XLIX. Over one hundred single-sporangium cultures were then made, and in addition a number of other types were isolated (Plate XLV). All these new forms were designated by Roman numerals. The original culture was given the numeral I, while II, III, IV, and V were applied to the four saltants. Numbers II, III, and V have remained constant, although some fluctuations have been noted, especially in II (Plate XLV). In no case, however, has II, III, or V given rise to either I or IV; nor has no. IV given rise to any other form than I.



Text figure 2 shows the descendants of a single-sporangium culture of type I. Each generation is six days old, and all cultures were kept under



TEXT FIG. 2. Chart showing numerous lines of descent from a single-sporangium culture of *P. parasitica-rhei* type I. The black dots represent colonies of type I; the circles indicate those of type IV. Note that for the first ten generations type IV predominates, after which the occurrence of the two types is of more nearly equal frequency.

TABLE II. *The Effect of M/100 Solutions of Phosphates on Sporangium-formation*

Organisms	Dihydrogen Potassium Phosphate	Sodium Phosphate	Ammonium Phosphate
<i>P. arecae</i> .....	I	II	II
<i>P. cactorum</i> 1.....	III	I	III
<i>P. cactorum</i> 2.....	III	I	III
<i>P. cactorum</i> 3.....	III	I	IV
<i>P. fagi</i> .....	III	III	III
<i>P. capsici</i> .....	II	III	II
<i>P. citrophthora</i> .....	I	II	III
<i>P. cinnamomi</i> .....	o	o	o
<i>P. cryptogea</i> .....	o	o	o
<i>P. erythroseptica</i> .....	o	o	o
<i>P. mexicana</i> .....	o	o	o
<i>P. infestans</i> .....	I	I	I
<i>P. phaseoli</i> .....	II	II	II
<i>P. nicotianae</i> .....	III	III	III
<i>P. syringae</i> .....	II	II	III
<i>P. parasitica</i> .....	IV	III	IV
<i>P. terrestris</i> .....	IV	III	IV
<i>P. sp.</i> (Reddick's) I.....	III	III	III
<i>P. sp.</i> (Reddick's) II.....	IV	IV	IV
<i>P. parasitica-rhei</i> I.....	III	III	III
<i>P. parasitica-rhei</i> II.....	III	III	IV
<i>P. parasitica-rhei</i> III.....	IV	IV	IV
<i>P. parasitica-rhei</i> IV.....	III	III	III

TABLE II (Continued)

Organisms	Dihydrogen Potassium Phosphate		Sodium Phosphate		Ammonium Phosphate	
<i>P. parasitica-rhei</i> V . . . . .	IV		IV		IV	
<i>P. faberi</i> . . . . .	III		III		III	
<i>P. palmivora</i> . . . . .	III		III		III	
<i>P. colocasiae</i> . . . . .	III		III		III	
139 . . . . .	IV		IV		IV	
142 . . . . .	IV		IV		IV	
143 . . . . .	III		IV		IV	
145 . . . . .	III		IV		IV	
123 . . . . .	IV		IV		IV	
126 . . . . .	III		IV		IV	
136 . . . . .	III		III		III	
127 . . . . .	IV		IV		IV	
137 . . . . .	IV		IV		IV	
138 . . . . .	III		III		III	
140 . . . . .	IV		IV		IV	
144 . . . . .	III		IV		IV	
22 . . . . .	III		III		IV	
26 . . . . .	II		II		II	
97 . . . . .	o-II		II		III	
100 . . . . .	o-III		II		III	
102 . . . . .	o-II		o-II		o-III	
117 . . . . .	o-II		o-II		o-III	
116 . . . . .	o-II		II		II	
141 . . . . .	III		IV		IV	
8 . . . . .	o		o		II	
36 . . . . .	o-II		o-II		o-I	
44 . . . . .	o		o-II		o-II	
121 . . . . .	o		o		o	
130 . . . . .	o-II		o-II		o-II	
372 . . . . .	Spor. I	Oög. III	Spor. I	Oög. II	Spor. III	Oög. III

identical environmental conditions. The black dots represent type I, while the circles designate type IV. It can be seen that type I in the second generation has split into I and IV. Cultures made from type IV, thus produced, split again, but from then on there is a line of type IV which constantly breeds true. All the remaining, however, either split continuously, or type IV may breed true for several generations and then split again, and type I may split after breeding true for three or four generations. It is not uncommon for one type to change over entirely into the other type. These phenomena suggest a possible segregation of hybrid characters, but an absence of pure line of type I, the fact that saltations did not arise until nearly six months after the culture of *P. parasitica-rhei* was obtained, and the subsequent results, which will be explained shortly, argue strongly against this assumption.

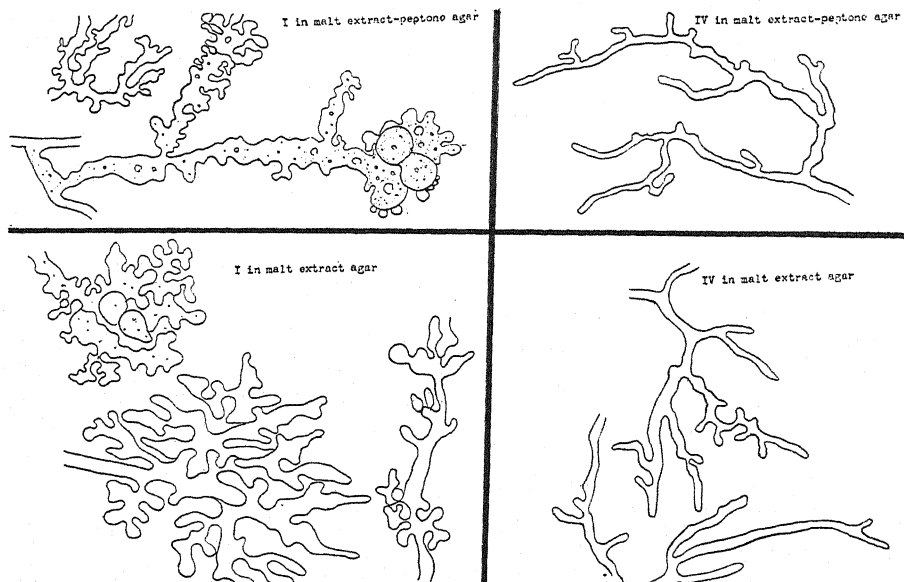
The following list gives a clear picture of the differences between types I and IV:

## Type I

1. Abundant aerial hyphae (Pl. XLIX).
2. Granular, lumpy growth of colonies (text fig. 3).
3. Gnarled, irregular, submerged hyphae (text fig. 3).
4. Abundant sporangia on solid agar.
5. Abundant oögonia and chlamydo-spores on oatmeal agar.
6. Large sporangia (text fig. 4).
7. Good quantity of sporangia formed when food is renewed daily.
8. Not saltating in sugars, ammonium chlorid, and ammonium sulfate.

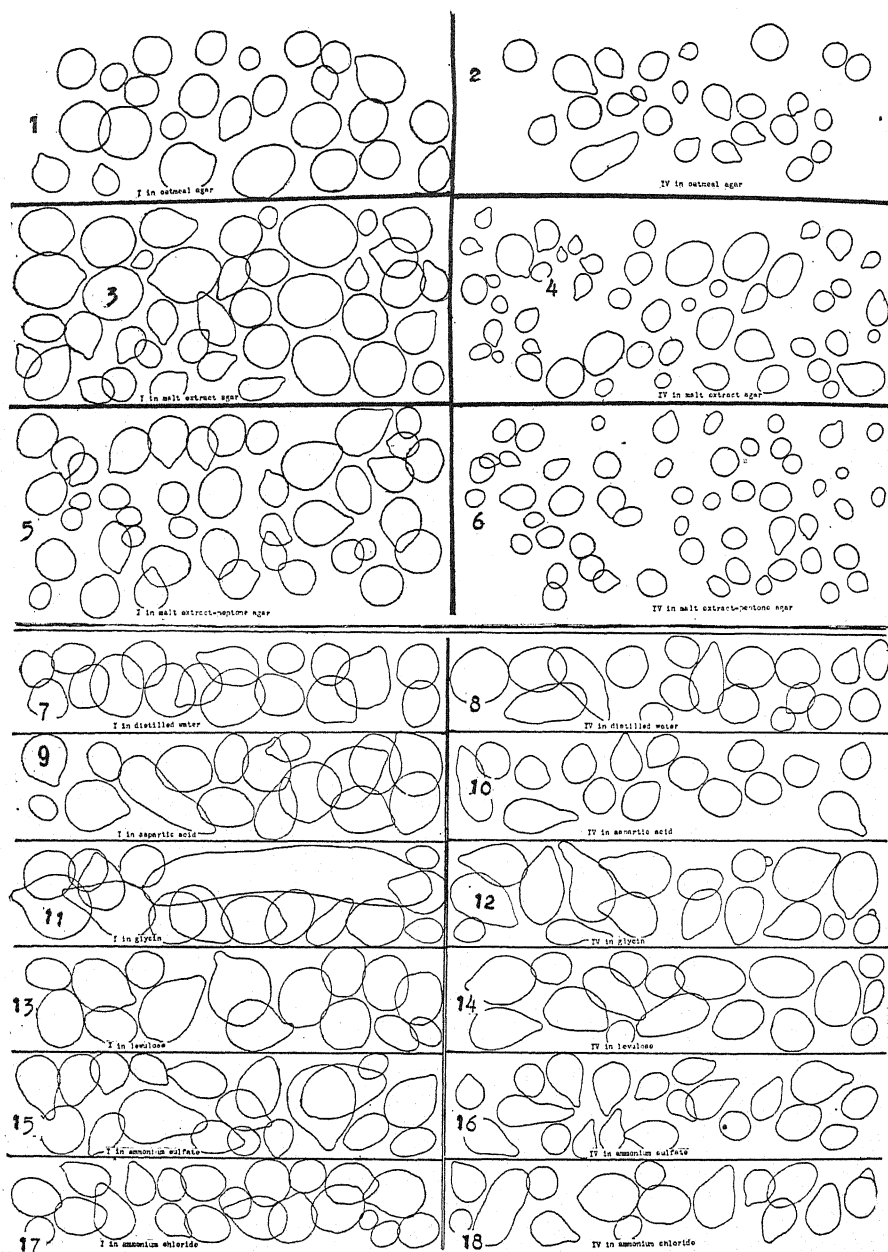
## Type IV

1. No aerial hyphae (Pl. XLIX).
2. Fine, even growth.
3. Fine, regular, submerged hyphae (text fig. 3).
4. Very few sporangia.
5. Very few oögonia and chlamydo-spores.
6. Sporangia much smaller (text fig. 4).
7. Remaining sterile.
8. Saltating.



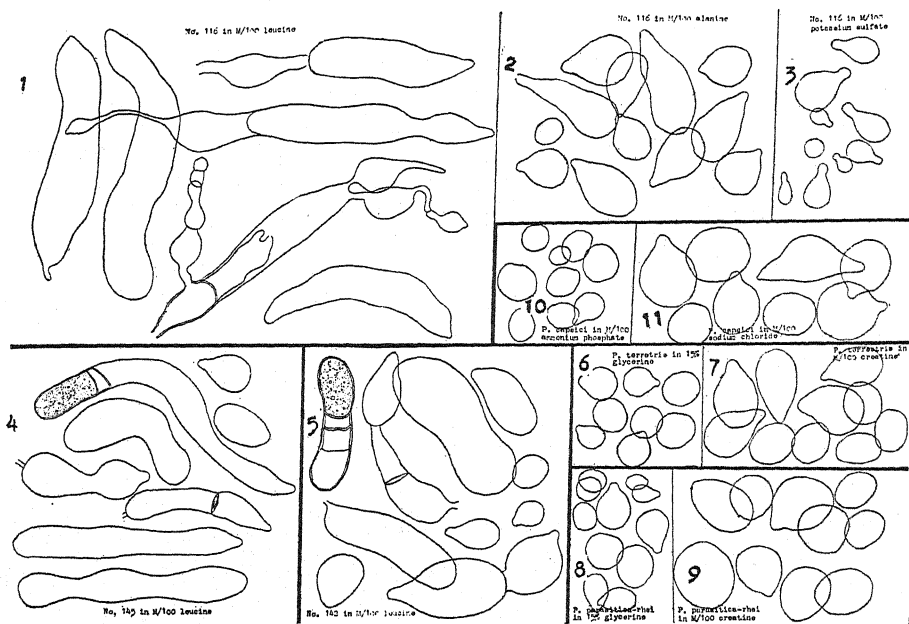
TEXT FIG. 3. The nature of the *submerged* mycelium of types I and IV. The left-hand column shows the hyphae of type I in malt-extract-peptone agar above, and in malt-extract agar below. The right-hand column represents the submerged hyphae of type IV in the same agars.

These differences are sufficient to justify making a new species of type IV. But fortunately the story does not end here. This supposedly pure line of type IV was found to be not so pure as it appeared; at any rate it is too complex, as can be seen from the following experiments: cultures of type IV from the pure line shown in text figure 2 were made in the nutrient solution, and the ensuing colonies were transferred to distilled water, glycine, glutamic acid, aspartic acid, alanine, levulose, etc. The resulting



TEXT FIG. 4. A comparison of the sporangia of *P. parasitica-rhei* types I and IV. The sporangia shown in the left column are of type I; those shown on the right are of type IV. 1 and 2 in oatmeal agar; 3 and 4 in malt-extract agar; 5 and 6 in malt-extract-peptone agar; 7 and 8 in distilled water; 9 and 10 in aspartic acid; 11 and 12 in glycine; 13 and 14 in levulose; 15 and 16 in ammonium sulfate; 17 and 18 in ammonium chloride.

sporangia were found to be quite as large as those formed by type I (text fig. 4). If the reversion stopped here we should still be justified in regarding this organism as a distinct form easily separated from type I. But it went much further. When the fungus was allowed to reproduce in the solutions mentioned and was then transferred to malt-extract plates, startling things began to happen; type IV, after breeding true for so many generations without manifesting any sign of reversion, split again and



TEXT FIG. 5. The effects of various substances upon the shape and size of sporangia. 1, no. 116 in M/100 leucine. This effect was obtained only once; another attempt under the same conditions yielded a few very small and regularly shaped sporangia; numerous other attempts gave negative results and the fungus remained sterile in this solution. 2, no. 116 in M/100 alanine. 3, no. 116 in M/100 potassium sulfate. 4, no. 145 in M/100 leucine. 5, no. 142 in M/100 leucine. 6, *P. terrestris* in 15 percent glycerine. 7, *P. terrestris* in M/100 creatine. 8, *P. parasitica-rhei* I in 15 percent glycerine. 9, *P. parasitica-rhei* I in M/100 creatine. 10, *P. capsici* in M/100 ammonium sulfate. 11, *P. capsici* in M/100 sodium chloride.

produced colonies part of which were composed of type I and another part of type IV, and thus the process illustrated in text figure 2 started anew. A more complete example of reversion could not be pictured. Roberts (6) has mentioned the fact that his saltations of *Alternaria mali* reverted back and forth for a time, but that they eventually established a pure-breeding line. Stevens (7) and others emphasize the production of saltations in their cultures; but reversions have received little or no attention, and no

one has reported a case in which a well established saltant was made to revert back to its original.

Old cultures of type IV descended from the pure line also show a tendency towards reversion. As stated before, the age of each generation of cultures shown in text figure 2 was only six days, and consequently the old-age factor was not materially involved. It is evident that two strains of type IV have been present; one of these splits into the two types regardless of the time factor, while the other is able to breed true so long as it is kept in a good growing condition. Probably this is one of the reasons why an apparently pure line was established. When this second strain was allowed to grow old in test tubes and its growth was arrested, or when its physiological balance was disturbed by being transferred to various solutions, type I again appeared. It should be noted in this connection that type IV remained sterile when the food was renewed daily. Sterility or scanty reproduction seems to be the chief characteristic of a strongly established race of type IV. This is at once apparent on solid media. The reverting phenomenon exhibits a struggle for expression between the

TABLE 12. *The Effect of M/100 Solutions of Sulfates on Sporangium-formation*

Organisms	Potassium Sulfate	Magnesium Sulfate	Ammonium Sulfate
<i>P. arecae</i> .....	I	I	o
<i>P. cactorum</i> 1.....	IV	IV	I
<i>P. cactorum</i> 2.....	IV	IV	I
<i>P. cactorum</i> 3.....	IV	III	I
<i>P. fagi</i> .....	IV	III	I
<i>P. capsici</i> .....	III	III	o
<i>P. citrophthora</i> .....	II	II	II
<i>P. cinnamomi</i> .....	o	o	o
<i>P. cryptogea</i> .....	o	o	o
<i>P. erythroseptica</i> .....	o	o	o
<i>P. mexicana</i> .....	o	o	o
<i>P. infestans</i> .....	III	II	I
<i>P. phaseoli</i> .....	II	II	II
<i>P. nicotianae</i> .....	III	III	II
<i>P. syringae</i> .....	III	II	II
<i>P. parasitica</i> .....	V	IV	IV
<i>P. terrestris</i> .....	III	IV	III
<i>P. sp.</i> (Reddick's) I.....	IV	IV	IV
<i>P. sp.</i> (Reddick's) II.....	IV	IV	IV
<i>P. parasitica-rhei</i> I.....	IV	V	IV
<i>P. parasitica-rhei</i> II.....	III	III	II
<i>P. parasitica-rhei</i> III.....	IV	IV	III
<i>P. parasitica-rhei</i> IV.....	III	III	o-III

TABLE 12 (Continued)

Organisms	Potassium Sulfate	Magnesium Sulfate	Ammonium Sulfate
<i>P. parasitica-rhei</i> V.....	III	III	II
<i>P. faberi</i> .....	III	IV	III
<i>P. palmivora</i> .....	III	III	II
<i>P. colocasiae</i> .....	III	II	I
139.....	IV	IV	III
142.....	IV	IV	I
143.....	III	III	II
145.....	IV	IV	II
123.....	IV	IV	IV
126.....	IV	III	II
136.....	III	III	II
127.....	IV	IV	III
137.....	IV	IV	III
138.....	IV	III	III
140.....	IV	III	III
144.....	III	III	II
22.....	III	III	o
26.....	III	II	o
97.....	III	II	o
100.....	III	II	o
102.....	II	II	o
117.....	II	II	o
116.....	II	I	o
141.....	III	II	I
8.....	I	I	o
36.....	o-II	I	o
44.....	II	o-II	o
121.....	o	o	o
130.....	o-II	o-II	o
372.....	Spor. III	Oög. III	Spor. o
			Oög. o

fertile and the sterile types in a composite form like *P. parasitica-rhei*. When the food is no longer renewed and the colonies are allowed to grow stale, the balance breaks up and the sterile or the scantily reproducing strain gives way to, or splits into, the other type.

It was stated that sporangia of type IV increased to normal size after the mycelium was transferred to the various solutions mentioned previously, and that a subsequent transfer to malt-extract agar induced a sudden splitting. It should not be assumed, however, that this is a regular phenomenon. The experiments were repeated a number of times, but the results were not uniform. At one time a large number of large sporangia would be formed but the colonies produced by these would fail to split, while at other times the sporangia failed to form in some of the solutions, or if formed would be extremely small and few in number, yet some of them would be able to give rise to splitting colonies. Since no consistent results were obtained, no generalizations or acceptable explanations can be given. Why two organisms, so unlike both physiologically and morphologically, should give rise to each other and then revert back again regardless

of environmental conditions, is more than can be explained at present. The only dependable treatment whereby type IV can be kept pure is a frequent transfer to fresh media or a daily renewal of food. The same can not be said, however, of type I. This is quite independent in its actions. A transfer made from a pure-breeding line of type IV gives rise to a pure colony of type IV; but a transfer from type I into a plate of agar may not only fail to produce a pure colony, but may even change over entirely into type IV. Old age, young and vigorous conditions, renewal of food, etc., seem to have no effect; yet when first obtained it bred true for nearly six months. While no explanation is offered as to the causal factor involved, it seems that an environmental rather than a hereditary factor was responsible. A similar phenomenon was more carefully followed in the case of no. 116. This organism behaved normally until it was grown on concentrated malt-extract agar, where the ensuing colony split into two distinct types (Pl. XLVIII, figs. 38, 41). Descendants of these showed a certain analogy to *P. parasitica-rhei*; the original continued to saltate, while the

TABLE 13. *The Effect of M/100 Solutions of Chlorids and a Carbonate on Sporangium-formation*

Organisms	Sodium Chlorid	Magnesium Chlorid	Ammonium Chlorid	Potassium Carbonate
<i>P. arecae</i> .....	II	II	I	II
<i>P. cactorum</i> 1.....	III	IV	III	IV
<i>P. cactorum</i> 2.....	III	IV	II	IV
<i>P. cactorum</i> 3.....	IV	IV	II	IV
<i>P. fagi</i> .....	III	IV	III	III
<i>P. capsici</i> .....	IV	III	III	II
<i>P. citrophthora</i> .....	III	III	II	I
<i>P. cinnamomi</i> .....	o	o	o	o
<i>P. cryptogea</i> .....	o	o	o	o
<i>P. erythroseplica</i> .....	o	o	o	o
<i>P. mexicana</i> .....	o	o	o	II
<i>P. infestans</i> .....	I	II	I	o
<i>P. phaseoli</i> .....	II	III	III	III
<i>P. nicotianae</i> .....	III	III	III	IV
<i>P. syringae</i> .....	III	III	o	III
<i>P. parasitica</i> .....	IV	IV	IV	IV
<i>P. terrestris</i> .....	IV	V	IV	IV
<i>P. sp.</i> (Reddick's) I.....	IV	V	IV	IV
<i>P. sp.</i> (Reddick's) II.....	IV	IV	IV	III
<i>P. parasitica-rhei</i> I.....	IV	IV	IV	III
<i>P. parasitica-rhei</i> II.....	III	IV	III	III
<i>P. parasitica-rhei</i> III.....	IV	IV	III	III
<i>P. parasitica-rhei</i> IV.....	III	III	o-III	III



TABLE 13 (Continued)

Organisms	Sodium Chlorid	Magnesium Chlorid	Ammonium Chlorid	Potassium Carbonate
<i>P. parasitica-rhei</i> V.....	IV	IV	III	III
<i>P. faberi</i> .....	IV	IV	III	IV
<i>P. palmivora</i> .....	IV	IV	III	III
<i>P. colocasiae</i> .....	III	III	III	III
139.....	IV	IV	III	III
142.....	IV	IV	III	IV
143.....	III	IV	III	III
145.....	IV	IV	II	III
123.....	IV	IV	IV	III
126.....	IV	III	II	IV
136.....	III	III	III	III
127.....	IV	III	III	IV
137.....	IV	V	III	III
138.....	IV	IV	III	IV
140.....	IV	III	II	IV
144.....	IV	IV	II	IV
22.....	IV	IV	o	II
26.....	III	II	o	I
97.....	II	II	o	III
100.....	II	II	o	II
102.....	II	II	o	I
117.....	II	II	o	II
116.....	II	II	I	II
141.....	III	II	II	II
8.....	o-II	o-I	o	o
36.....	III	o-II	o	I
44.....	II	II	o	o-III
121.....	o	o	o	o-II
130.....	o-II	o-II	o	o-II
372.....	Spor. I Oöğ. III	Spor. II Oöğ. III	Spor. o Oöğ. o	Spor. I Oöğ. III

new type continued to breed true. The saltation was not confined to the concentrated medium, but appeared on the ordinary malt-extract agar as well. Apparently the high food content was the chief factor in the disturbance of the physiological balance.

It may now be inquired if all organisms are not capable of splitting up under the proper stimulus. The plant and animal world is full of evidences supporting the possibility of such a phenomenon. Most of these evidences have been ascribed to segregations of hybrid characters. But what about so-called vegetative sports? What about bacteria and fungi which do not possess a sexual phase? Löhnis (4, 5), Stevens (7), and others list a large number of cases, both from their own observations and from those of others, showing that many organisms can change over or split into other forms. It is very likely that many investigators have discarded some interesting saltations thinking that these were contaminations. The so-called conflicting results obtained in the laboratories and ascribed to faulty methods, in spite of well controlled environmental conditions, may be nothing less than reverting saltations. Many of the negative results,

neglected or discarded by the workers bent upon obtaining nothing but positive results, were possibly cases of saltations.

It will be interesting to determine what is responsible for saltations. An effort was made so to change the nature of malt-extract agar as to check the tendency of splitting in the case of type I. Consequently, some malt-extract agar was made and was divided into seventeen portions. Each portion received equal amounts (equivalent to M/10 concentration) of one of the following substances: levulose, sucrose, glucose, glycine, leucine, alanine, asparagin, creatin, saccharin, one percent aminoids, one percent peptone, calcium nitrate, potassium nitrate, magnesium sulfate, magnesium chlorid, and dihydrogen potassium phosphate. The checks consisted of malt-extract agar only. Type I was transferred to these agars, and a few days later it was seen that in the checks, the mineral salts, the sugars, and the saccharin all the cultures either split or reverted to type IV, and in no case was type I able to overcome type IV. In agars to which amino acids, and especially alanine, were added, only 50 percent of the cultures split, and in the remaining cultures type I usually outgrew type IV. An addition of one percent peptone gave the most outstanding results; in about 80 percent of the cultures type I did not split, and in the other 20 percent it outgrew type IV. One would be led to conclude that a peptone agar would further suppress the splitting tendency. But such was not the case, as the majority of the colonies did split on this agar. The same thing happened when an alanine agar was tried. Apparently there is a balance somewhere, and type I might be made to remain constant if the proper combination of food factors were known.

#### THE PHYSIOLOGICAL STATUS OF PHYTOPHTHORA SPECIES

Tables 1-13 indicate a strong tendency among the various organisms to group themselves into distinct units. While this group solidarity has broken up from time to time to bring out some specific distinctions, a large number of the organisms, nevertheless, have remained so close together that no clear-cut physiological basis can be offered for their separation. The specific validity of such forms is not, therefore, recognized in this work. They may possess some sort of morphological distinction, but such is not as yet apparent, and any one insisting on their specific integrity should furnish more data than we now possess.

*P. cactorum* and *P. fagi* are very closely and most unmistakably related. There is no clear-cut break in the continuity of their physiological similarity to enable the worker to separate them with any degree of certainty. The few dissimilarities consist of differences in the amount of fruiting bodies rather than of the presence or absence of these. The morphological features are still more obscure, since they rest on such a hair-splitting basis as a difference of three microns in the sizes of oögonia and sporangia of *P. cactorum* and *P. fagi*. It seems best, therefore, to include *P. fagi* in *P.*

*cactorum* and thus to eliminate a superfluous species, although Himmelbaur and others recognize *P. fagi* as a definite species. But since the only important differences seem to be in the size of reproductive bodies and in the parasitic habits of the two fungi, and since these differences are too slight to be of specific value, *P. fagi* is hereby eliminated.

Even the status of *P. cactorum* is not as substantial as could be desired. There are too many similarities between *P. cactorum* and *P. parasitica* and comparatively few dissimilarities. The ease and abundance of oögonial reproduction in *P. cactorum* and the unfavorable effect of aspartic acid, glutamic acid, and ammonium sulfate (tables 9, 12) constitute the chief points of division between *P. cactorum* and *P. parasitica*. A close examination of these two tables shows that the differences mentioned are not so formidable as they seem. Other strains of *P. parasitica* are also unfavorably affected by ammonium sulfate, and nearly all the strains are, at times, just as negative in their reactions towards these two amino acids as *P. cactorum*. What is more, the periodicity in this respect is more pronounced in some strains. For example, *P. parasitica*, *P. terrestris*, *P. parasitica-rhei*, and Reddick's organism have manifested a definite cycle in their reactions toward these two amino acids. Beginning with the summer of 1923 and until the spring of 1924, these organisms gave negative results whenever they were transferred to either aspartic or glutamic acid. In the spring of 1924, however, sporangia began to form regularly in these solutions. Then they appeared more irregularly again and fluctuated between negative and positive results, but generally speaking the positive reactions predominated. It can not be said that an acid-tolerating strain was developed because reversions have occurred from time to time. The foregoing chart (text fig. 2) is too expressive to encourage such a notion. It is not at all impossible, therefore, that some strains of *P. cactorum*, or the present strains in some future experiments, when the investigator is fortunate enough to encounter the other periodic cycle (provided, of course, that such a cycle exists in this organism), will form sporangia in these amino acids. As stated before, the differences in the size of sporangia are of no consequence. *P. parasitica-rhei* IV has very much smaller sporangia than *P. parasitica-rhei* I, yet it can not be made a new species. No. 144 has much smaller sporangia than most of the strains of *P. parasitica*, yet it is merely a strain.

*P. nicotianae* is another organism the taxonomic status of which is far from being definitely established. It resembles *P. parasitica* too closely, both in its morphological as well as in most of its physiological characteristics. Its negative reaction towards aspartic acid, glutamic acid, and some of the sugars, as well as its apparent inability to produce sporangia when transferred daily to a fresh supply of nutrient solution, or to a concentrated nutrient solution (table 5), serve to keep it distinct from *P. parasitica*. The writer regrets that he was unable to secure a variety of strains of this fungus to test more thoroughly the sphere of its reactions.

While *P. capsici* and *P. citrophthora* exhibit a number of close similarities, they possess many clear-cut dissimilarities. The same statement applies to *P. erythrosepica* and *P. mexicana*, although to a much less degree. Perhaps two species and two varieties should be made of these four species, but this will serve no practical purpose and it is as confusing to have new varieties as to have new species. If one were to attack the taxonomy of the genus *Phytophthora* from an academic viewpoint, he would have little difficulty in demonstrating that all its species are so closely related as properly to be combined into one. This, however, would not help the taxonomist, and the average worker would be too much at sea concerning the identity of his organisms.

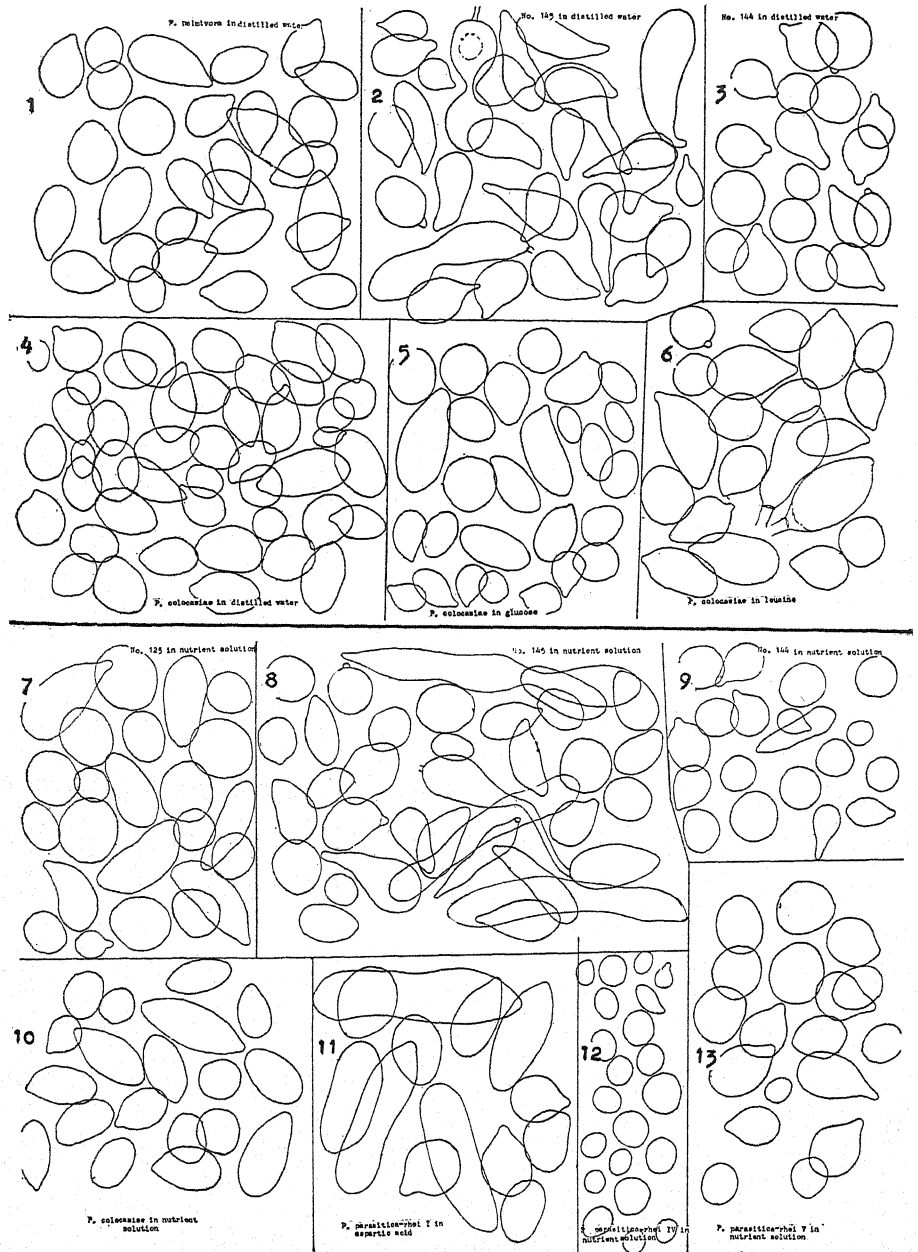
The resemblance between *P. infestans* and *P. phaseoli* is too striking to be overlooked. There is no doubt in the writer's mind that these two can not be placed in different species, consequently *P. phaseoli* is made a variety of *P. infestans*. The chief value of a variety is its usefulness in emphasizing a distinct relationship. *P. infestans*, to be sure, produces oögonia very rarely, at least most of the time. But the same phenomenon is illustrated in some saltants; *P. parasitica-rhei* I forms oögonia while *P. parasitica-rhei* III does not, and *P. parasitica-rhei* IV produces them very rarely. Is the formation of a new species justified because of these oögonial differences? *P. parasitica-rhei* IV is much more clearly differentiated from *P. parasitica-rhei* I than is *P. phaseoli* from *P. infestans*.

The 24 organisms listed in the foregoing tables from *P. parasitica* to no. 144 inclusive are so closely related that not more than one species is there represented. These forms can not be separated either physiologically or morphologically. While some of them may be recognized by certain minor characteristics, such as the quantity of aërial hyphae, nature and rate of colony growth, presence and absence of oögonia, etc., these characteristics are not dependable. Unless substantiated by some outstanding and more stable specificity rather than such meager and misleading points, they can possess no great taxonomic value. The fact that no oögonia have been seen in *P. faberi* is not a specific characteristic. Gadd (1) has demonstrated heterothallism in this organism. He found that strains from cacao and papaw constitute one group, and those from Hevea, Dendrobium, etc., form the other. When grown apart from each other these strains are unable to produce oögonia. When, however, they are grown together, oögonia are produced in abundance. Throughout the thirteen tables it can be seen that *P. faberi* and *P. palmivora* are almost identical in their reactions, and there is no possibility of adequately separating them. The following eight organisms, nos. 123, 126, 136, 127, 137, 138, 140, and 141, are identical with *P. faberi* and *P. palmivora*. Nos. 139, 142, 143, and 145 are identical with *P. parasitica*. The differences between *P. parasitica* and *P. palmivora* or *P. faberi* are too slight to warrant a specific segregation. A typical colony of *P. parasitica* has a granular, lumpy growth on malt-

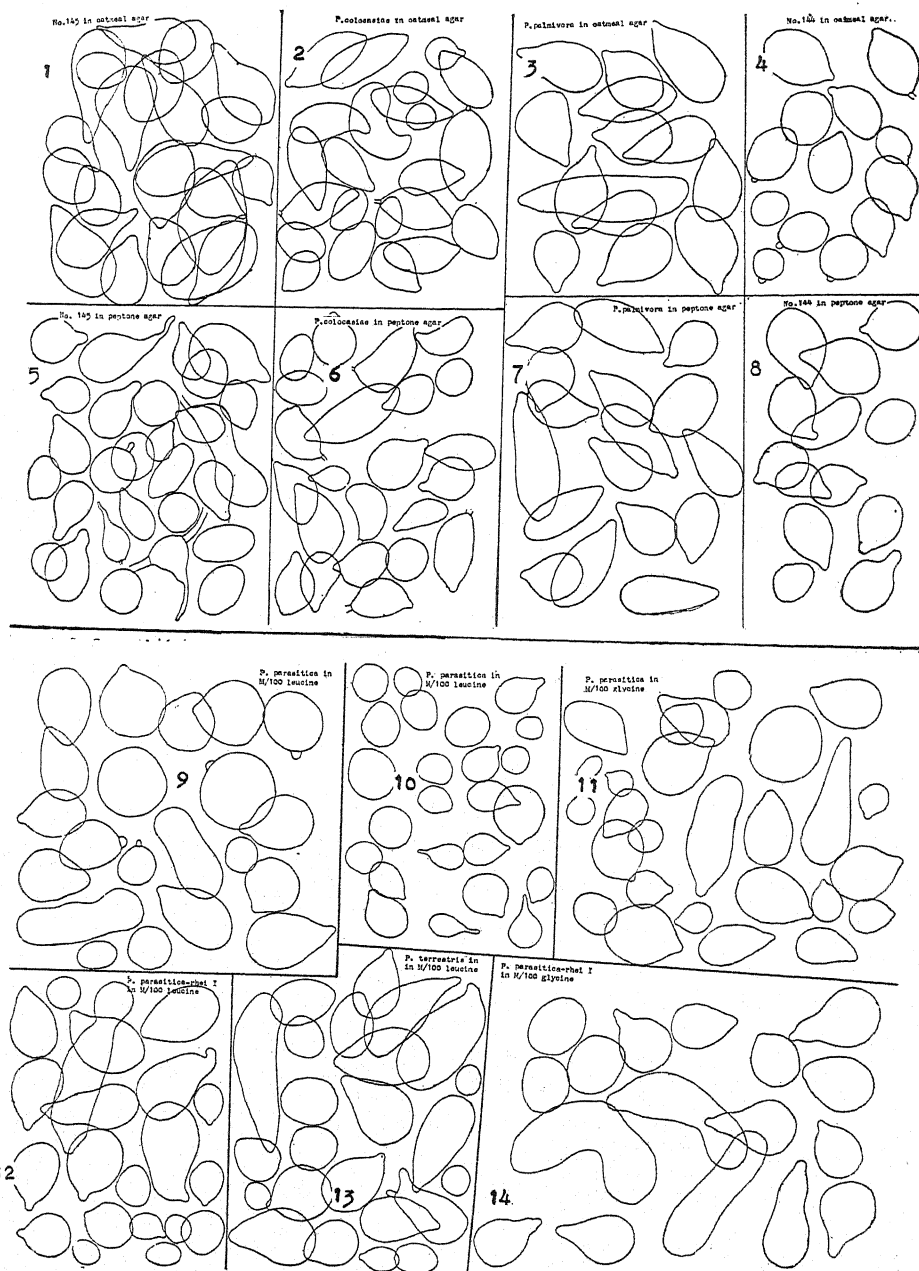
extract, nucleinic-acid, and other transparent agars (Pl. XLV), whereas colonies of *P. faberi* or *P. palmivora* are homogeneous, having a smooth-combed appearance (Pl. XLV, XLVI). It has also been shown that *P. parasitica* var. *rhei*, which is nothing more than *P. parasitica*, yields a saltant, type V, which produces typical *P. faberi* colonies (Pl. XLV). Other saltants of *P. parasitica-rhei* (types III and IV) yield growth characteristics which bear no similarity to those of a typical *P. parasitica* colony. The submerged hyphae of typical *P. parasitica* forms are gnarled, irregular, greatly enlarged, while those of *P. palmivora*, *P. faberi*, etc., are smooth and even (text fig. 3), yet *P. parasitica-rhei* IV and V possess even and smooth hyphae. *P. parasitica* forms produce a dark gray ring just below the slant of two-months-old oatmeal-agar tubes, while *P. faberi* and *P. palmivora* fail to give rise to such a ring. Yet *P. parasitica-rhei* IV also fails in this respect. *P. parasitica* strains produce oögonia in oatmeal and other agars, *P. faberi* and *P. palmivora* not at all or only under certain conditions; yet *P. parasitica-rhei* III also fails to form oögonia. Lastly, typical colonies of *P. parasitica* grow slowly, while colonies of *P. faberi* and *P. palmivora* grow more rapidly. Yet *P. sp.* (Reddick's) II, which is also a strain of *P. parasitica*, grows much faster than *P. faberi* or *P. palmivora*.

We can, in a general and arbitrary way, divide these 24 organisms into two sub-groups: (a) *Parasitica*, and (b) *Colocasiae*. Sub-group *a* includes *P. parasitica*, *P. terrestris*, Reddick's organism and its saltant, *P. parasitica-rhei* and its saltants, nos. 139, 142, 143, and 145. Sub-group *b* embraces *P. faberi*, *P. palmivora*, *P. colocasiae*, nos. 123, 126, 136, 127, 137, 138, 140, and 144. These two sub-groups come under one species, or perhaps one species group, for which DeBary's *P. omnivora* is reestablished as the most appropriate and the earliest specific name. Since *P. cactorum* and *P. fagi* have long ago been segregated from *P. omnivora*, there is no reason why this name should be discarded for newer and less appropriate names.

The chief characteristic ascribed to *P. colocasiae* is the fact that its sporangia are definitely longer than those of *P. parasitica*. If this were a well established morphological feature, the writer would hesitate to include *P. colocasiae* in *P. omnivora* and would perhaps make it a new variety. But, as a matter of fact, this organism forms a large number of spherical sporangia in addition to the more elongated and the larger ones (text fig. 6). *P. palmivora* also produces a large number of elongated sporangia. So do the other members of the group (text figs. 5-7), although not all are identical in this respect. The shape and size of sporangia are so extremely variable that it is unwise to retain a species because of such a slight and unstable morphological feature. Nor can we make host relationships a safe criterion in taxonomic work. In the light of our present knowledge of biologic forms, the fact that *P. colocasiae* appears to be confined to *Colocasia esculenta*, whereas other species of *Phytophthora* are unable to attack this plant, can not be of specific significance.



TEXT FIG. 6. Variability of sporangia within the same strain as well as in other strains, further demonstrating that the sizes and shapes of these bodies are not reliable for specific distinctions. 1, *P. palmivora* in distilled water. 2, no. 145 in distilled water. 3, no. 144 in distilled water. 4, *P. colocasiae* in distilled water. 5, *P. colocasiae* in *M*/100 glucose. 6, *P. colocasiae* in *M*/100 leucine. 7, no. 123 in the nutrient solution. 8, no. 145 in the nutrient solution. 9, no. 144 in the nutrient solution. 10, *P. colocasiae* in the nutrient solution. 11, *P. parasitica-rhei* I in aspartic acid. 12, *P. parasitica-rhei* IV in the nutrient solution. 13, *P. parasitica-rhei* V in the nutrient solution.



TEXT FIG. 7. The variability of sporangia under the same conditions, showing that the shapes and sizes of sporangia can not be specific characteristics. 1, no. 145 in oatmeal agar. 2, *P. colocasiae* in oatmeal agar. 3, *P. palmivora* in oatmeal agar. 4, no. 144 in oatmeal agar. 5, no. 145 in peptone agar.<sup>3</sup> 6, *P. colocasiae* in peptone agar. 7, *P. palmivora* in peptone agar. 8, no. 144 in peptone agar. 9, *P. parasitica* in M/100 leucine. 10, *P. parasitica* in M/100 leucine (conditions in these experiments were identical and the size of these fungi). 11, *P. parasitica* in M/100 glycine. 12, *P. parasitica-rhei* I in M/100 leucine. 13, *P. terrestris* in M/100 leucine. 14, *P. parasitica-rhei* I in M/100 glycine.

<sup>3</sup> Peptone agar: Dihydrogen potassium phosphate 0.6 g., magnesium sulfate 0.3 g., peptone 1 g., maltose 5 g., agar 20 g., distilled water 1000 cc.

The hosts of *P. parasitica* are so diverse and so widely scattered that the existence of numerous biological forms is not at all improbable. Tomatoes, rhubarb, and tobacco in the United States, Bryophyllum in Bermuda, Hibiscus and Abaca in the Philippines, cacao in Java, castor bean in India, etc., as host plants for this organism give an idea of its cosmopolitan and omnivorous habit.

The cumulative value of such secondary characteristics as the shape and size of sporangia, the presence and absence of aërial hyphae and oögonia, the rate and the nature of colony growth, etc., is not at all disputed; their specific value, however, is not recognized unless there are other and more definite points of differentiation. This seems to be the only safe procedure, otherwise great confusion will arise. If, for example, it is assumed that the sub-group Parasitica consists of one distinct species, and sub-group Colocasiae of another, then what will be done with *P. parasitica-rhei* IV? It belongs to the first sub-group since it arose from a member of that group, yet it does not fit there but rather in the sub-group Colocasiae. Can it be said that one species gave rise to another one? Even if such a looseness be allowed in the conception of the species, how will the saltating habit of this organism be explained? Shall an entirely different species be made of it, or shall it be said that during a part of its life cycle it is one species, and at another time a different one?

Nos. 22, 26, 97, 100, 102, 117, 116, and 141 seems to constitute a transition group. There is no doubt that during one phase of their life cycle these organisms definitely belong to *P. omnivora*, but at another time or under different conditions they exhibit reactions which are foreign to typical strains of *P. omnivora*. The two phases in their life cycle do not follow one another very regularly and even seem to occur independently of outside conditions. One of the peculiar characteristics of sub-group Parasitica is that the pure hyphal colonies, when transferred to leucine, may sometimes yield dark-brown sporangia, and even the mycelium may become colored, so much so that the entire culture appears dark gray or even blackish. No. 116 usually fails to reproduce in leucine; once, however, it formed a few sporangia, but these bodies showed no discoloration and no distortion of shape. A second trial yielded a large number of sporangia, most of them with the shapes and sizes shown in text figure 5. Soon the entire culture turned dark brown. A large number of subsequent trials failed to duplicate this condition, and the fungus remained sterile in leucine.

Similar instances have been observed in forms of *P. omnivora*. In the fall of 1923, whenever the mycelium of *P. parasitica*, *P. terrestris*, *P. parasitica-rhei*, or Reddick's organism was transferred to M/100 leucine solution, the cultures became mahogany brown and the sporangia were greatly enlarged and abnormally elongated. When this process was repeated in the spring of 1925, the mycelium remained white and the sporangia, especially in the case of *P. parasitica*, remained abnormally small. Transfers



were made three more times, but the sporangia were always very small (text fig. 6) and only once did the size of sporangia revert to the larger types. It is the same process as that exhibited by no. 116, the same periodicity, and the same uncertain reactions. Academically speaking, no. 116 and the organisms grouped with it can not be considered other than strains of *P. omnivora*, although for practical purposes the formation of a new variety would be the more desirable solution.

Numbers 8, 36, 44, 121, and 130 differ so sharply from all others and from one another as to make them an isolated group with at least two or three distinct forms.

Now is the time to ask again: What constitutes a species? Undoubtedly a species is not and never can be a rigid mold into which all members must either fit perfectly or be fitted to other molds. It is unreasonable to expect two members of the same species to appear alike and to behave identically even under controlled conditions. Minor differences will always exist. There may be extremes of these differences, and one or more members may possess a part or the majority of these extremes, whereas another member may possess only a few or the opposite extremes; one member may be more strongly pronounced in some features, and another member less so, while still others may follow a middle course. But the strains of a species group exhibit so many and so close similarities that they should be grouped together in spite of their minor differences. If a few of the reactions of the *Omnivora* group are tabulated here, the force of foregoing statements will appear distinctly.

Rate of growth in malt-extract agar at 20° C.:

20-35 mm. *P. parasitica*, *P. terrestris*, *P. parasitica-rhei* II, III, IV, nos. 143, 139.

40-55 mm. *P. sp.* (Reddick's) I, *P. parasitica-rhei* I and V, *P. faberi*, *P. palmivora*, *P. colocasiae*, nos. 142, 145, 123, 136, 127, 140, 144.

60-75 mm. *P. sp.* (Reddick's) II, nos. 126, 137.

Aërial hyphae on malt-extract agar at 20° C.:

o. *P. parasitica-rhei* III and IV.

I. *P. faberi*, nos. 145, 136, 137.

II. *P. parasitica-rhei* II and V, *P. colocasiae*, nos. 139, 127, 140, 144.

III. *P. parasitica*, *P. terrestris*, *P. sp.* (Reddick's) I, *P. palmivora*, nos. 142, 123, 126, 138.

IV. *P. sp.* (Reddick's) II, *P. parasitica-rhei* I, no. 143.

Aërial hyphae on concentrated malt-extract agar at 20° C.:

o. *P. parasitica*, *P. terrestris*, *P. sp.* (Reddick's) I, *P. parasitica-rhei* II and III, *P. faberi*, *P. palmivora*, nos. 142, 143, 145, 123, 136, 127, 137, 140, 144.

- I. No. 139.
- II. *P. parasitica-rhei* I, IV, V, *P. colocasiae*.
- III. *P. sp.* (Reddick's) II, nos. 126, 138.

Sporangia on malt-extract agar at 20° C.:

- I. *P. parasitica-rhei* IV.
- II. *P. colocasiae*, no. 144.
- III. *P. parasitica-rhei* II, III, V, *P. faberi*, *P. palmivora*, nos. 139, 142, 143, 145, 136, 140.
- IV. *P. parasitica*, *P. terrestris*, *P. sp.* (Reddick's) I, *P. parasitica-rhei* I, nos. 123, 126, 137.
- V. *P. sp.* (Reddick's) II, nos. 127, 138.

Sporangia formed after transfer to 2 cc. of nutrient solution:

- II. *P. parasitica-rhei* IV, *P. palmivora*.
- III. *P. parasitica*, *P. terrestris*, *P. sp.* (Reddick's) I and II, *P. parasitica-rhei* I, II, III, V, *P. faberi*, *P. colocasiae*, nos. 139, 143, 126, 136, 137.
- IV. Nos. 142, 145, 123, 127, 138, 140, 144.

Sporangia formed after transfer to dextrose:

- III. *P. sp.* (Reddick's) II, *P. parasitica-rhei* II, III, *P. faberi*, *P. palmivora*, *P. colocasiae*, nos. 143, 145, 123, 126, 136, 138.
- IV. *P. parasitica*, *P. terrestris*, *P. sp.* (Reddick's) I, *P. parasitica-rhei* I, III, II, V, nos. 139, 142, 127, 137, 144.

Sporangia formed after transfer to sodium chlorid:

- III. *P. parasitica-rhei* II, IV, *P. colocasiae*, nos. 143, 136.
- IV. *P. parasitica*, *P. terrestris*, *P. sp.* (Reddick's) I and II, *P. parasitica-rhei* I, III, V, *P. faberi*, *P. palmivora*, nos. 139, 142, 145, 123, 126, 127, 137, 138, 140, 144.

An examination of the foregoing list shows that, if rate of growth were a specific characteristic, the members of the Omnivora group would constitute at least three distinct species. For example, *P. parasitica-rhei* II, which formed a colony not larger than 20 mm. in diameter, would not be considered the same organism as *P. parasitica-rhei* I, which grew twice as fast, and *P. sp.* (Reddick's) II would be distinct from both of the former because it grew very much faster than either of them.

A classification based on aërial hyphae would break the group into five parts; nor would the production of aërial hyphae on concentrated malt-extract agar simplify the situation. Sporangium-formation on the solid agar fails to offer a better solution. The combination of these three different reactions would make the situation less chaotic, to be sure, but still would leave it too mixed to be of great help to the taxonomist. However, when we begin to consider the liquid cultures, larger and fewer groups are seen

to evolve, and the extremes gradually disappear until in sodium-chlorid solution only two closely related groups remain. Add to these facts the reactions toward a great number of other solutions, and the sum total of the averages points to only one species group. The taxonomic situation thus clarifies, while the biological situation commands more attention.

The natural conclusion is, therefore, that type species should be discarded and species groups should be given prominence if a hopelessly tangled taxonomic situation is to be avoided. The species group should be large enough to accommodate all forms which possess a large number of similar characteristics. It should not be so small as to exclude organisms which may exhibit certain minor peculiarities and modifications of their own, nor so large as to include such forms as have too many specific and consistent qualities. The conception that the members of a given species should resemble each other like two drops of water is dangerous. It tends to make the investigator a slave to type species which are collecting dust and degenerating in various herbaria, not to speak of the multiplication of worthless species the number of which is already assuming alarming proportions.

Such minor characteristics as the size and shape of fruiting bodies, the rate and nature of growth, presence and absence of aërial hyphae and oögonia, should not be disregarded. They have their place in any scheme of classification, but it should be a secondary place. The average of all the morphological, pathological, and physiological features should form the specific sphere. Care should be taken, however, to avoid indefinite and uncertain situations. The border line between species must be as nearly clear-cut as possible if confusion is to be eliminated.

While the minor characteristics mentioned should claim only a secondary place in systematic work, biologically they are of great importance since they serve as distinctive milestones in the evolution of organisms. In different strains or in fixed saltations in which these minor qualities do appear and breed true, we come face to face with a situation that demands attention. Whether we call these saltants, strains, forms, or types, they should be tagged with some mark of identification, even if it be only a number, because today's strain may be tomorrow's variety and the species of the near or distant future. But so long as no new and major specific points appear, there will be no necessity of adding new species and thus creating confusing situations for those who are not vitally interested in the fascinating phenomenon of slow evolution, who do not care to split hairs over minor differences, and who are not sufficiently expert to see these differences or properly to interpret them, but who are concerned primarily with a safe classification of a given organism.

Nos. 8, 36, 44, 121, and 130 as a group have consistently stood apart from the other organisms and should be classified under a new species. These five forms grow alike, possess uniformly similar sporangia and hyphae,

and exhibit such a large number of similarities and so few dissimilarities that it seems unwise to make more than one new species. Nos. 36, 44, and 130 should undoubtedly be classified as one species, while nos. 8 and 121 can readily be made into new physiological forms. No attempt is made to name these, since Hartley has isolated and studied them in their native habitat and is better qualified to do so.

No. 372 is undoubtedly a new species for which the name of *P. pini* is proposed.

*Phytophthora pini* n. sp. Sporangia large, terminal, rarely intercalary, usually ovate and papillate, but spherical, oblong, and plano-convex forms not uncommon; papillae well defined or blunt, sometimes lacking altogether; mean length of sporangia 55.5 microns, mean width 35 microns, varying from 26–92.5 x 22–44.5 microns; oögonia formed in solid agars, but *also abundantly in nutrient solution* (see text for formula) *and when mycelium is transferred to M/100 sugars or even to distilled water*; mean size of oögonia 29 microns, varying from 18.5 to 37 microns. Antheridia both paragynous and amphigynous, more commonly paragynous, usually only one antheridium on each oögonium, but *two, three, and even four antheridia may be attached to the same oögonium*; *both types of antheridia on the same oögonium not uncommon*; antheridia originating either on the oögonial branch or developed in great numbers on distinct antheridial hyphae.

Weak parasite on roots of *Pinus resinosa* in Minnesota.

#### THE PHYSIOLOGICAL KEY

This key is based largely upon physiological reactions. It is not offered as the only solution for the taxonomic puzzle of the genus *Phytophthora*, since morphological and pathological features should also be considered. It is true that most, if not all, of the morphological characters are highly variable; it is equally true, however, that the physiological reactions, especially when the saltation phenomenon occurs, are also quite unstable; nor do host relationships simplify the situation. As stated before, the average of morphological, pathological, and physiological characteristics should constitute the sphere of a species. Any organism which exhibits the majority of the characteristics of a given species should be classified under that species group regardless of a few differences in morphological or physiological points.

It should be stated, however, that the physiological key offered here is workable so far as the particular organisms and the environmental conditions of this laboratory are concerned. The fact that some 26 unknown strains sent by Hartley were identified and classified a few weeks after being received, and that in spite of their widely separate origins they showed such close reactions, speaks favorably for the key. Undoubtedly it should be added to and improved upon from time to time, as it is only a working nucleus and is given as such rather than as a finished product. It should not be forgotten that a key is not a solution by itself; it is merely an indicator, a means to an end, and before any final identification is made,

therefore, the more detailed specific reactions listed in the foregoing tables should be consulted.

Inclusion of nos. 121, 8, 130, 26, and 116 in this key does not at all signify that these are definite species, but that they can be separated without difficulty from the other organisms and from one another. Since they all belong to the two most persistently saltating groups, it is probable that their apparent distinctive reactions will not always hold. However, until this fact is demonstrated experimentally, they can be included in the key.

A PHYSIOLOGICAL KEY FOR THE IDENTIFICATION OF  
PHYTOPHTHORA SPECIES<sup>4</sup>

1. Forming no colonies on malt-extract agar plates (six days at 20° C.).....(2)
2. No oögonia in oatmeal agar or lima-bean agar; no sporangia in potassium carbonate or in arginine..... 1. *P. infestans*.
2. Oögonia in oatmeal agar or lima-bean agar; sporangia in potassium carbonate or in arginine..... 2. *P. infestans* var. *phaseoli*.
1. Forming colonies on malt-extract-agar plates.....(3)
3. Sporangia produced in aspartic acid or glutamic acid.....(4)
4. No sporangia in nutrient solution.....(5)
5. Sporangia in nucleic-acid agar; no sporangia in ammonium nitrate..... 3. No. 26.
5. No sporangia in nucleic-acid agar; sporangia in ammonium nitrate..... 4. No. 116.
4. Sporangia in nutrient solution..... 5. *P. omnivora*.
3. No sporangia formed in aspartic or glutamic acid.....(6)
6. Oögonia in malt-extract agar (test tubes at 20° C. for one month).....(7)
7. No sporangia in leucine or ammonium sulfate; oögonia in levulose or potassium nitrate..... 6. *P. pini*.
7. Sporangia in leucine or ammonium sulfate; no oögonia in levulose or potassium nitrate..... 7. *P. cactorum*.
6. No oögonia in malt-extract agar.....(8)
8. No sporangia in nutrient solution.....(9)
9. Chlamydospores in nutrient solution; only chlamydospores in potassium nitrate or solid agars..... 8. *P. cinnamoni*.
9. No chlamydospores in nutrient solution or potassium nitrate.....(10)
10. No sporangia in potassium nitrate.....(11)
11. Oögonia in oatmeal agar; no sporangia in malt-extract agar or potassium carbonate..... 9. *P. cryptogea*.
11. No oögonia in oatmeal agar; sporangia in malt-extract agar or in potassium carbonate..... 10. No. 121.
10. Sporangia in potassium nitrate.....(12)
12. No sporangia in sodium chlorid.....(13)
13. Oögonia in calcium-nitrate agar; no sporangia in glycine. 11. *P. erythroseptica*.
13. No oögonia in calcium-nitrate agar; sporangia in glycine. 12. *P. mexicana*.
12. Sporangia in sodium chlorid.....(14)
14. No sporangia in mannose.....(15)

<sup>4</sup> In this key I is considered the same as O.

15. Sporangia in distilled water and in leucine.....13. *P. nicotianae*.
15. No sporangia in distilled water and in leucine.....14. No. 8.
14. Sporangia in mannose.....(16)
16. No sporangia in distilled water.....15. No. 130.
16. Sporangia in distilled water; sporangia in calcium nitrate.....(17)
17. Oögonia in oatmeal agar; no sporangia and no aërial hyphae in nucleinic-acid agar.....16. *P. capsici*.
17. No oögonia in oatmeal agar; sporangia and aërial hyphae in nucleinic-acid agar.....17. *P. citrophthora*.
8. Sporangia in nutrient solution.....(18)
18. No growth in malt-extract-agar plate at 12° C. (six days), good growth at 30° C.; no sporangia in ammonium sulfate.....17. *P. arecae*.
18. Growth in malt-extract-agar plate at 12° C., no growth at 30° C.; sporangia in ammonium sulfate.....18. *P. syringae*.

## SUMMARY

1. Fifty-three strains of *Phytophthora*, consisting of most of the described species of this genus, have been studied in pure culture.

2. Solid agars, liquid media, and M/100 solutions of a number of sugars, amino acids, nitrates, sulfates, phosphates, chlorids, and a carbonate have been used under controlled conditions to obtain certain specific reactions.

3. The presence and absence of aërial hyphae, of sporangia and oögonia, the rate and the type of growth of colonies on solid agars at different temperatures, and the production or non-production of sporangia and oögonia in the various solutions have enabled the writer to obtain a number of specific reactions which are useful in the identification of these organisms.

4. Some changes in the taxonomy of the genus are made: *Pythiacystis citrophthora* has been transferred to the genus *Phytophthora* under the name of *P. citrophthora*. It is proposed to make *P. phaseoli* a variety of *P. infestans*. *P. colocasiae*, *P. palmivora*, *P. faberi*, *P. parasitica*, *P. terrestris*, Reddick's *Phytophthora*, and *P. parasitica* var. *rhei* have been replaced by DeBary's *P. omnivora*.

5. The saltation phenomenon in *Phytophthoras* has been studied in detail, and it is concluded that no new species or varieties have been produced by saltation, and that, while the rate and type of colony growth, the shape and the size of sporangia, and the presence or absence of oögonia are greatly influenced by saltation, these characteristics are too unstable and reversible to be given primary specific importance.

6. *P. pini* is the name proposed for one of the new species included among the organisms here studied.

7. A key, based upon physiological reactions, is given.

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## EXPLANATION OF PLATES

## PLATE XLV

The habit of colony growth on malt-extract agar

- FIG. 1. No. 138.
- FIG. 2. *Phytophthora* sp. (Reddick's) I.
- FIG. 3. *P. parasitica*.
- FIG. 4. No. 139.
- FIG. 5. *Phytophthora* sp. (Reddick's) II.
- FIG. 6. *P. terrestris*.
- FIG. 7. *P. parasitica-rhei* IV.
- FIG. 8. *P. parasitica-rhei* III.
- FIG. 9. *P. parasitica-rhei* I.
- FIG. 10. *P. parasitica-rhei* V.
- FIG. 11. *P. parasitica-rhei* II.
- FIG. 12. *P. parasitica-rhei*-II. This shows the range of variation in the strains of this particular saltant. Photos by W. E. Rumsey.

## PLATE XLVI

The habit of colony growth on malt-extract agar (continued)

- FIG. 13. No. 123.
- FIG. 14. No. 145.
- FIG. 15. No. 140.
- FIG. 16. No. 126.
- FIG. 17. *P. palmivora*.
- FIG. 18. No. 142.
- FIG. 19. No. 127.
- FIG. 20. *P. faberi*.
- FIG. 21. No. 143.
- FIG. 22. No. 136.
- FIG. 23. *P. colocasiae*.
- FIG. 24. No. 144.

## PLATE XLVII

The habit of colony growth on malt-extract agar (continued)

- FIG. 25. *P. arecae*.
- FIG. 26. *P. capsici*.
- FIG. 27. *P. fagi*.
- FIG. 28. *P. syringae*.
- FIG. 29. *P. citrophthora*.
- FIG. 30. *P. cactorum* I.

- FIG. 31. *P. erythroseptica*.  
 FIG. 32. *P. cinnamoni*.  
 FIG. 33. *P. cactorum* 2.  
 FIG. 34. *P. mexicana*.  
 FIG. 35. *P. cryptogea*.  
 FIG. 36. *P. cactorum* 3.

## PLATE XLVIII

The habit of colony growth on malt-extract agar (continued)

- FIG. 37. *P. nicotianae*.  
 FIG. 38. No. 116-I.  
 FIG. 39. No. 22.  
 FIG. 40. No. 8.  
 FIG. 41. No. 116-II.  
 FIG. 42. No. 26.  
 FIG. 43. No. 8 on concentrated malt-extract agar.  
 FIG. 44. No. 116 on concentrated malt-extract agar.  
 FIG. 45. No. 97.  
 FIG. 46. *P. pini*.  
 FIG. 47. *P. pini* on concentrated malt-extract agar.  
 FIG. 48. No. 100.

## PLATE XLIX

Saltation of *P. parasitica* var. *rhei*.

- FIG. 1. The typical colony (a) forming the saltant (b).  
 FIG. 2. A typical colony before saltating.  
 FIG. 3. A typical colony of the saltant.

## PLATE L

Behavior of different sectors of the same colony

FIG. 1. A colony of an unstable strain of the saltant (type IV), showing the different sectors (a, b, c, d) from which transfers were made.

FIGS. 2 a, 3 d, 4 b, 5 c. The colonies formed when corresponding sectors from the plate shown in figure 1 were transferred to malt-extract agar.

## PLATE LI

Behavior of different sectors of the same colony (continued)

FIG. 6. A colony of the original culture (type I), showing the different sectors from which transfers were made.

FIGS. 7 e, 8 f, 9 g. The colonies formed when corresponding sectors from the plate in figure 6 were transferred to malt-extract agar.

## PLATE LII

Nature of submerged hyphae in different agars

A. Malt-extract agar.

B. Aminoids agar (aminoids 1 g., dihydrogen potassium phosphate 0.6 g., magnesium sulfate 0.3 g., dextrose 3.5 g., agar-agar 20 g., distilled water 1,000 cc.).

- FIGS. 1, 14. *P. parasitica*.  
 FIGS. 2, 15. *P. terrestris*.  
 FIGS. 3, 16. *P. parasitica-rhei*.  
 FIGS. 4, 17. *P. nicotianae*.



- FIGS. 5, 20. Reddick's Phytophthora.  
FIGS. 6, 18. *P. faberi*.  
FIGS. 7, 19. *P. fagi*.  
FIGS. 8, 21. *P. cryptogea*.  
FIGS. 9, 22. *P. cactorum*.  
FIGS. 10, 23. *P. capsici*.  
FIGS. 11, 24. *P. palmivora*.  
FIGS. 12, 25. *P. syringae*.  
FIGS. 13, 26. *P. citrophthora*.

## PLATE LIII

## Saltation of organism no. 126

- FIG. 1. The typical growth of no. 126 (a), giving rise to a typical growth of no. 136 (b).  
FIG. 2. No. 136 on concentrated malt-extract agar.  
FIG. 3. No. 126 on concentrated malt-extract agar.

## PLATE LIV

## The effect of temperature on the nature of colonies

- FIG. 1. No. 141 grown on malt-extract agar at 12° C.  
FIG. 2. The same organism on the same agar but at 30° C.  
FIG. 3. The same as the foregoing two but at 20° C.  
FIG. 4. The same as the foregoing three but grown alternately at 12° C. and 30° C.

## PLATE LV

## The effect of temperature (continued)

- FIG. 5. Type IV of *P. parasitica-rhei* grown alternately at 12° C. and 20° C.

## PLATE LVI

The effect of various substances upon the saltating habit of *P. parasitica-rhei*

- FIG. 1. Type I on malt-extract agar plus potassium phosphate.  
FIG. 2. Type I on malt-extract agar plus calcium nitrate.  
FIG. 3. Type I on malt-extract agar.  
FIG. 4. Type I on malt-extract agar plus magnesium chlorid.  
FIG. 5. Type I on malt-extract agar plus calcium nitrate.  
FIG. 6. Type I on malt-extract agar plus magnesium sulfate.  
FIG. 7. Type I on malt-extract agar plus peptone.  
FIG. 8. Type I on potassium nitrate agar.  
FIG. 9. Type I on malt-extract agar plus alanine.  
FIG. 10. Type IV on malt-extract agar plus peptone.  
FIG. 11. Type IV on potassium-nitrate agar.  
FIG. 12. Type IV on malt-extract agar plus alanine.

## PLATE LVII

*Phytophthora pini* n. sp.

## Sporangia and oögonia

- FIG. 1. Antheridial branches.  
FIGS. 2-5. Early stages in the development of antheridia and oögonia.  
FIGS. 6-11. Slightly older stages of antheridia and oögonia.  
FIGS. 12-20. Still older stages.

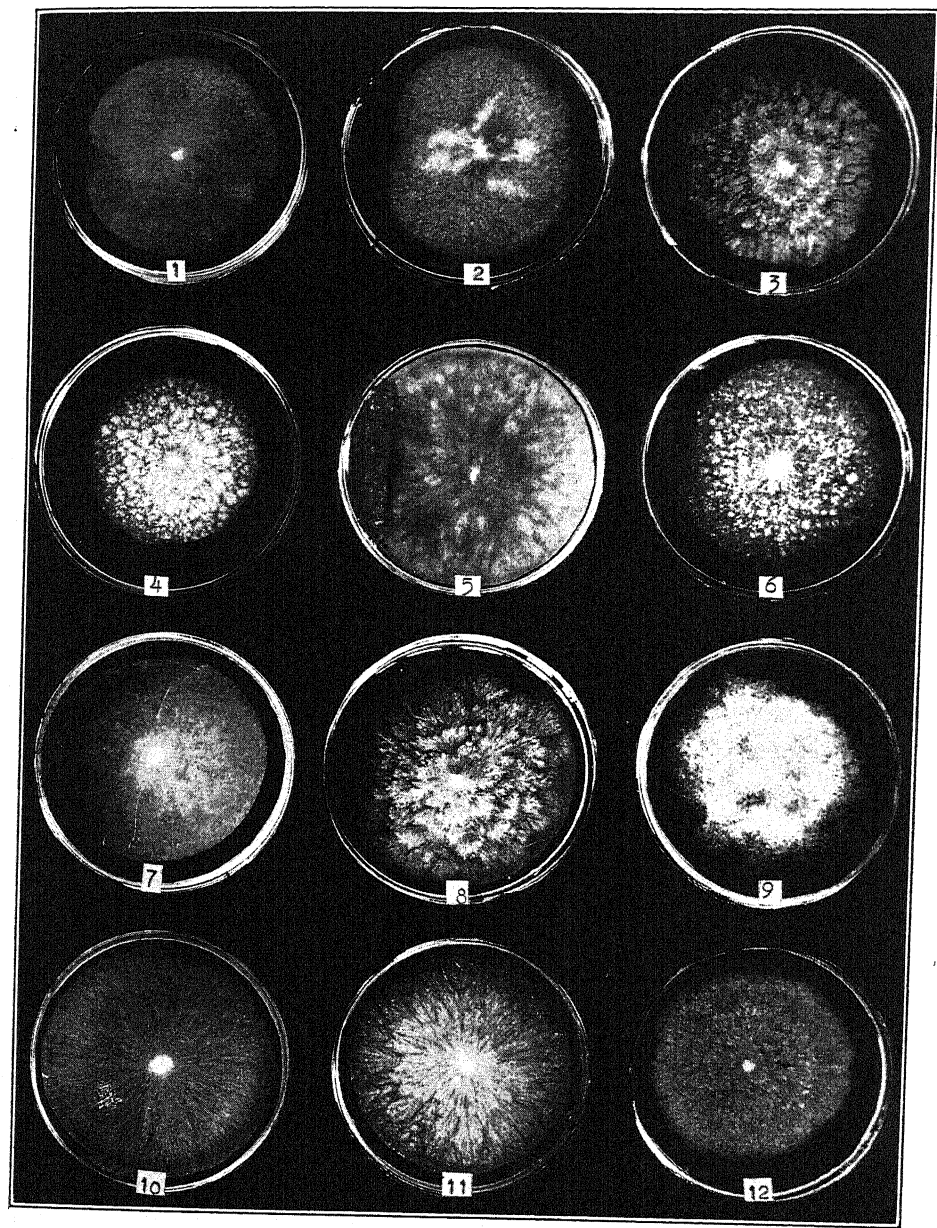
FIG. 21. Mature oögonium.

FIG. 22. Oöspore germinating.

In figure 9 the antheridium, after fertilizing (?) the oögonium, gives rise to a young antheridium at *a*, while another antheridium at *b*, after failing to come in contact with an oögonium, germinates to form two hyphae.

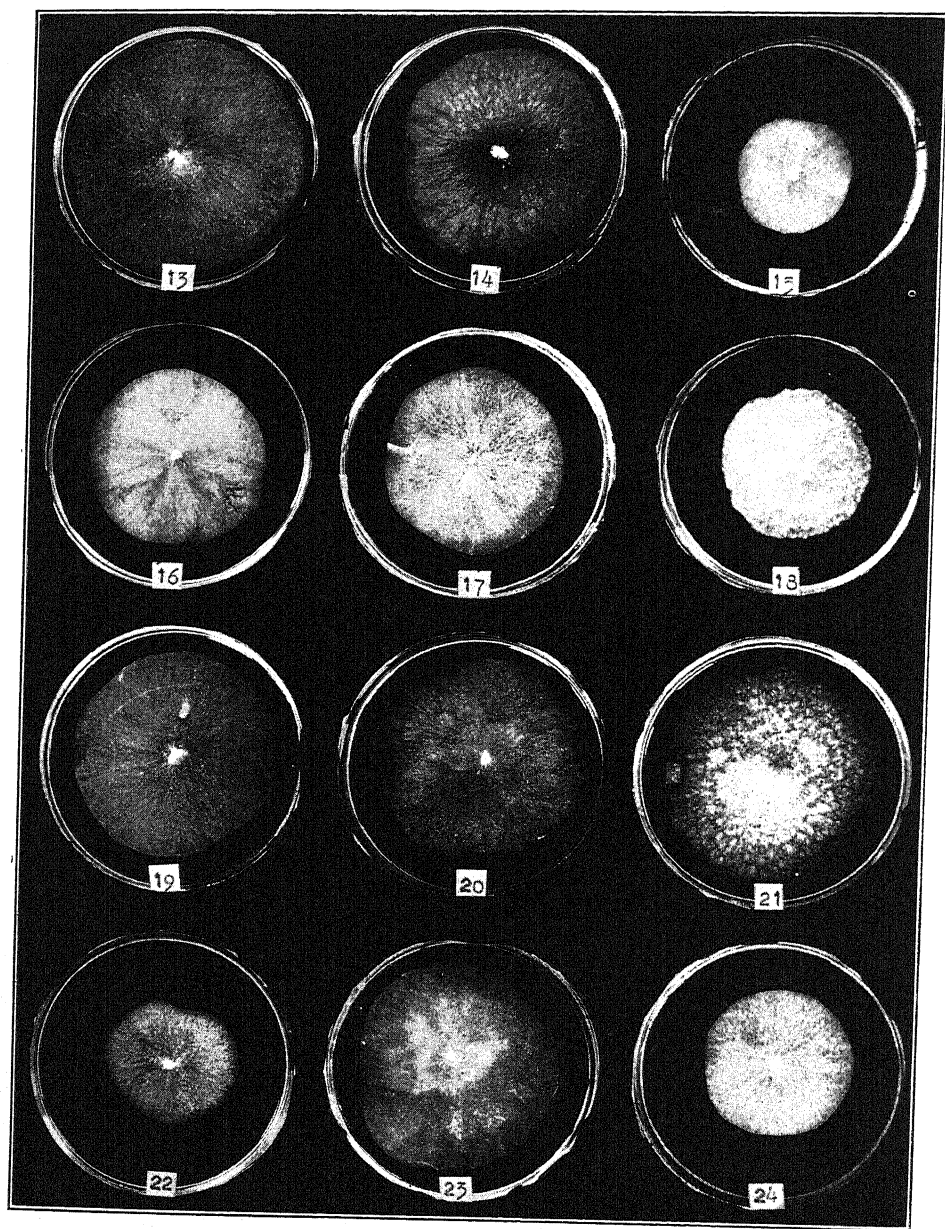
Figures 19 and 20 show the two types of antheridia on the same oögonium; figure 18 illustrates a case in which four antheridia have been attracted to one oögonium.

These figures were drawn with the aid of a camera lucida. The sporangia were obtained from a 24-hour water culture. The oögonia developed in a hanging drop of calcium-nitrate solution to which a vigorously growing but sterile mycelium was transferred.



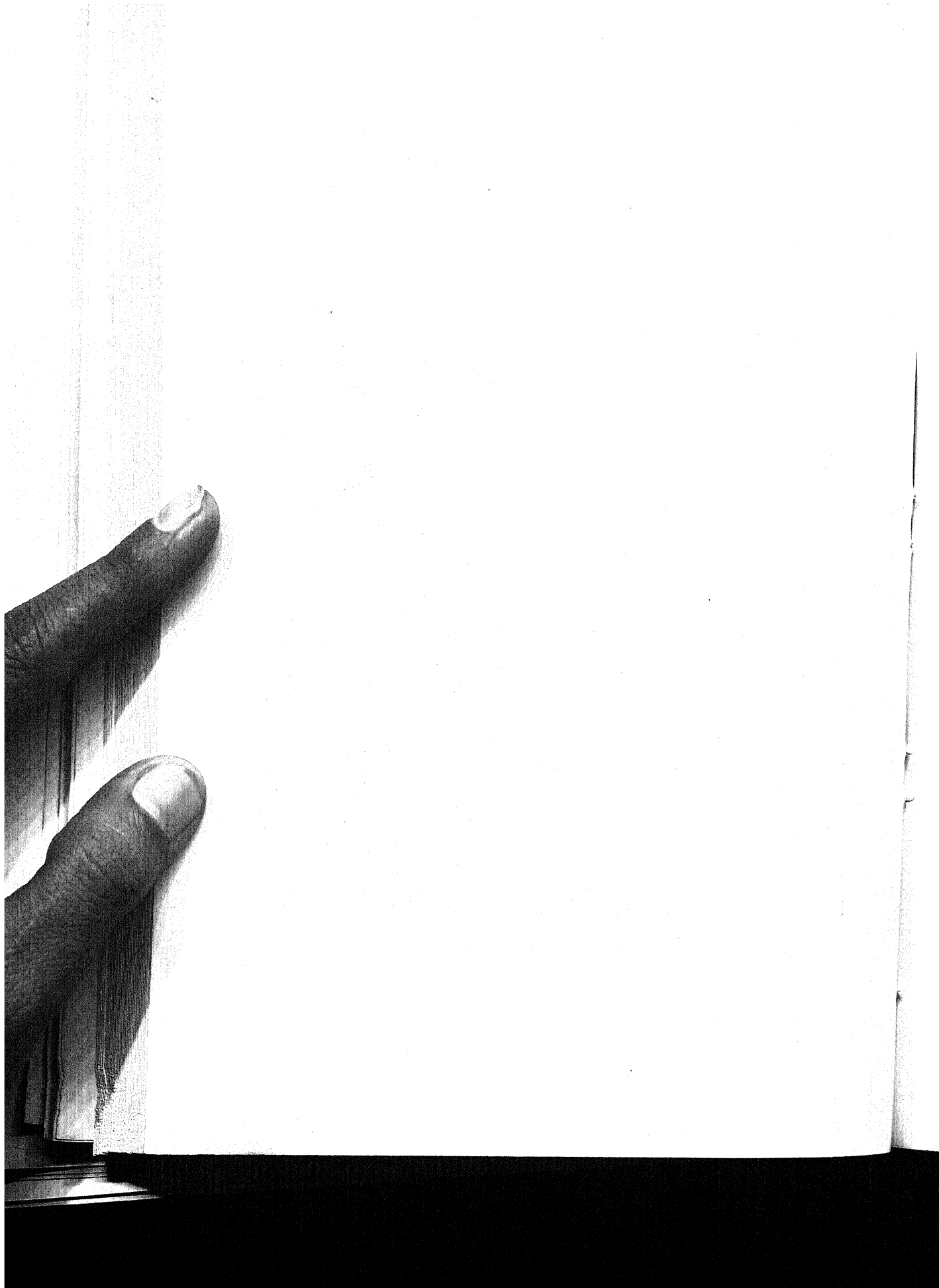
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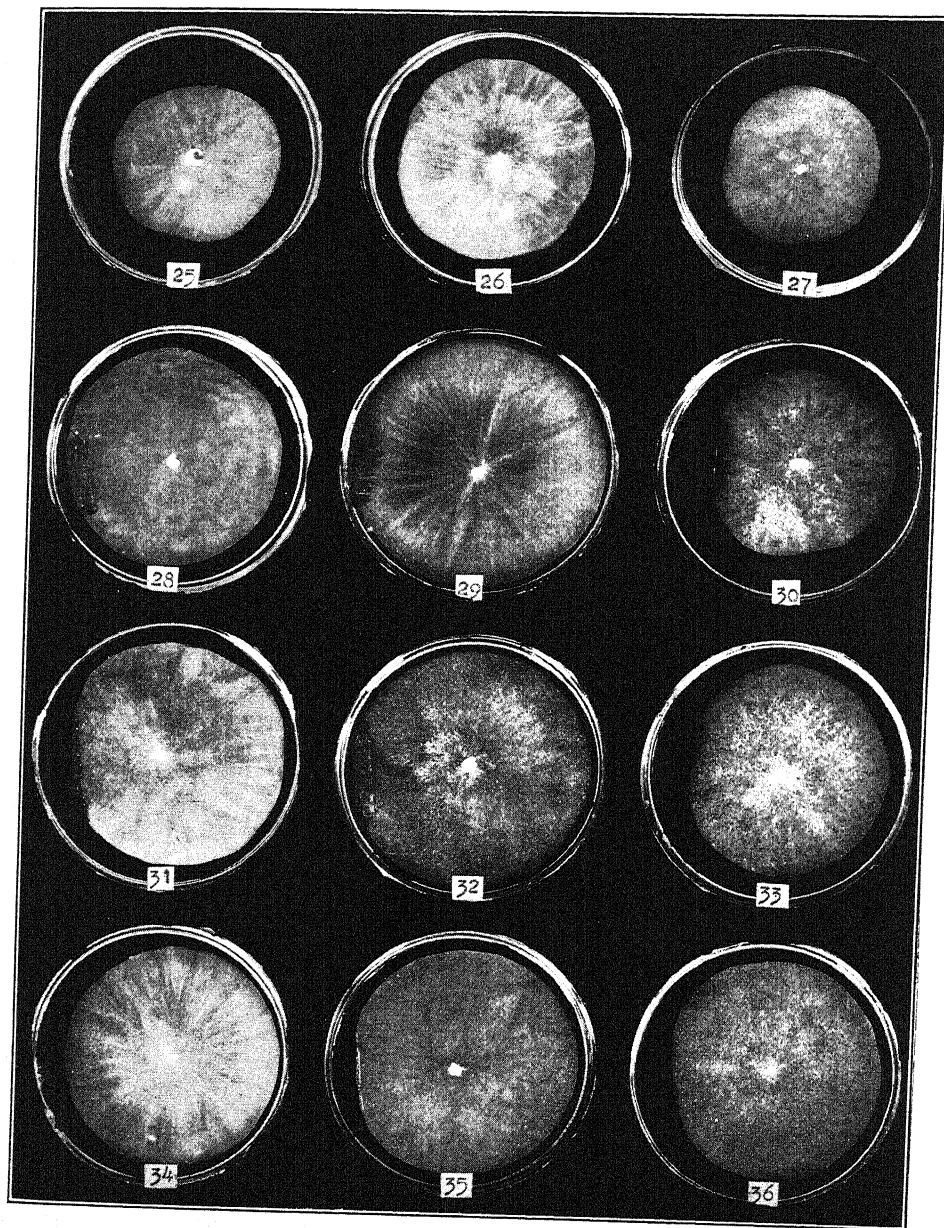


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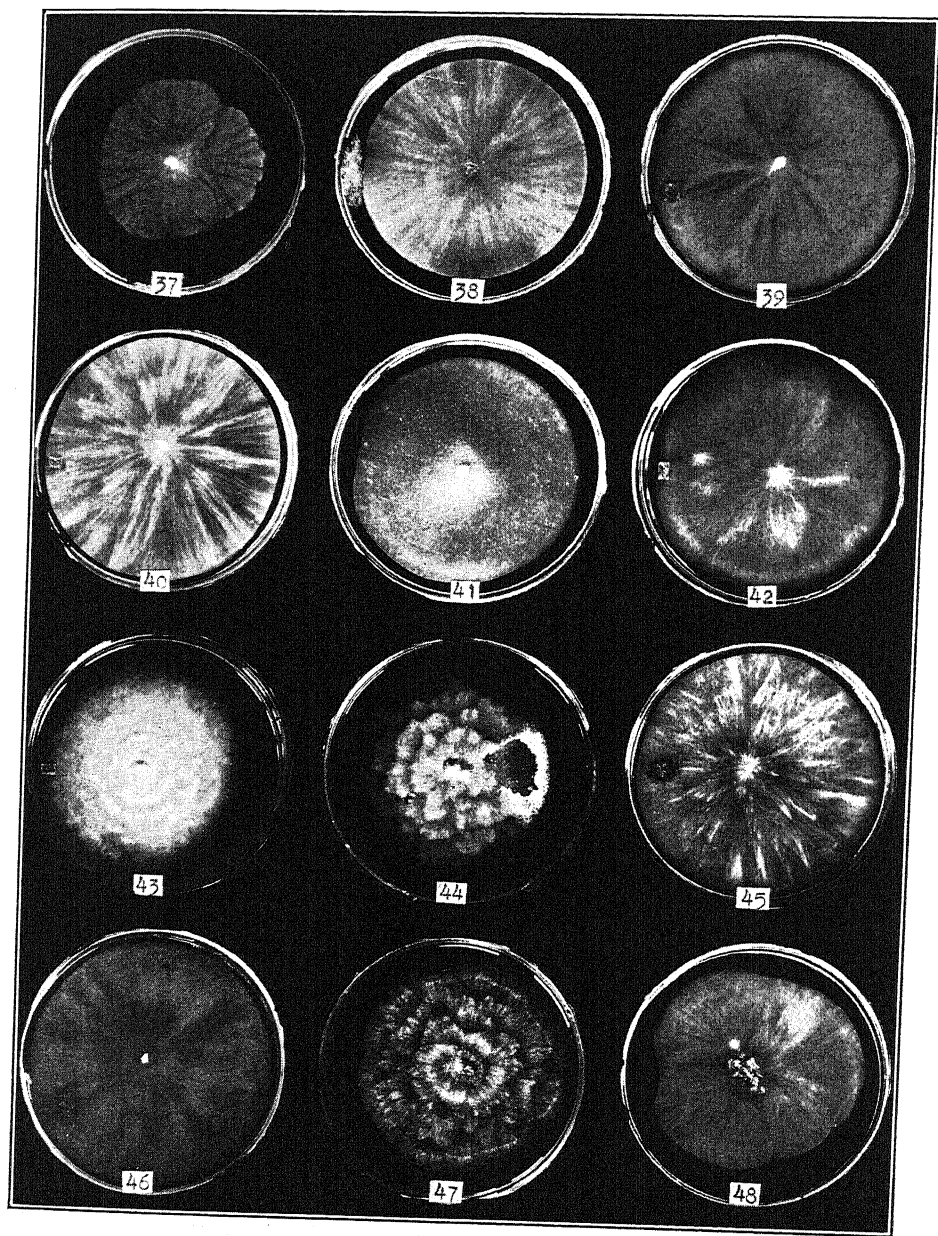


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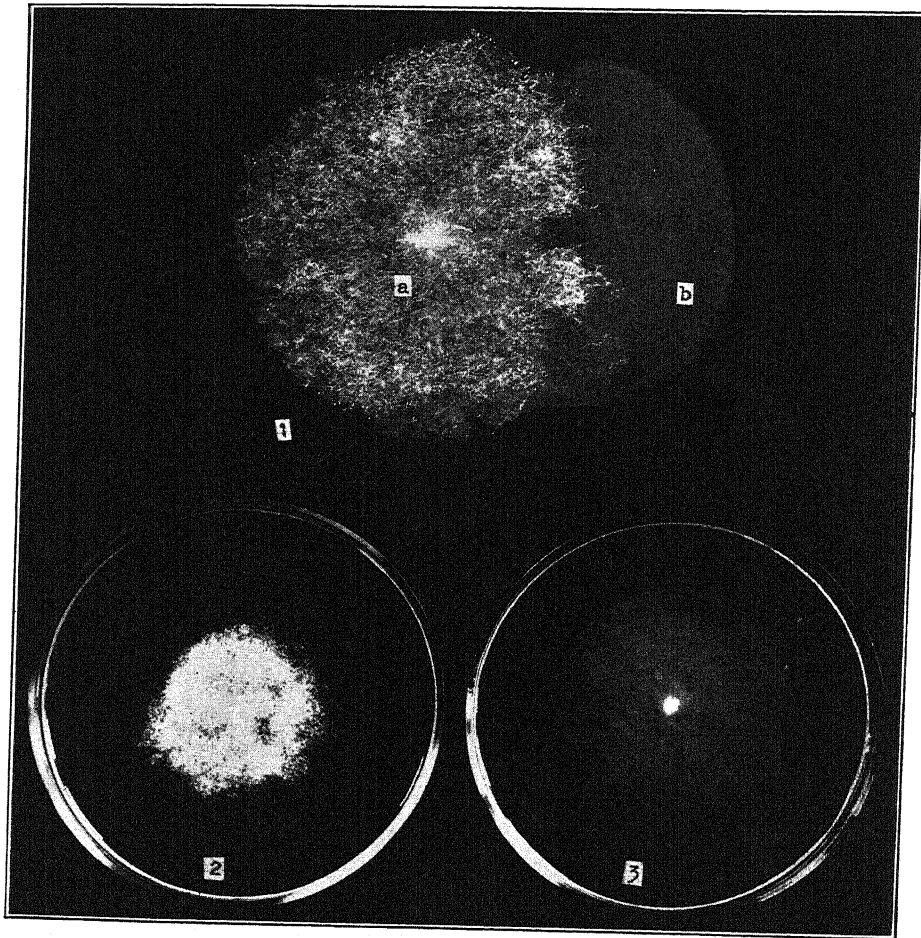




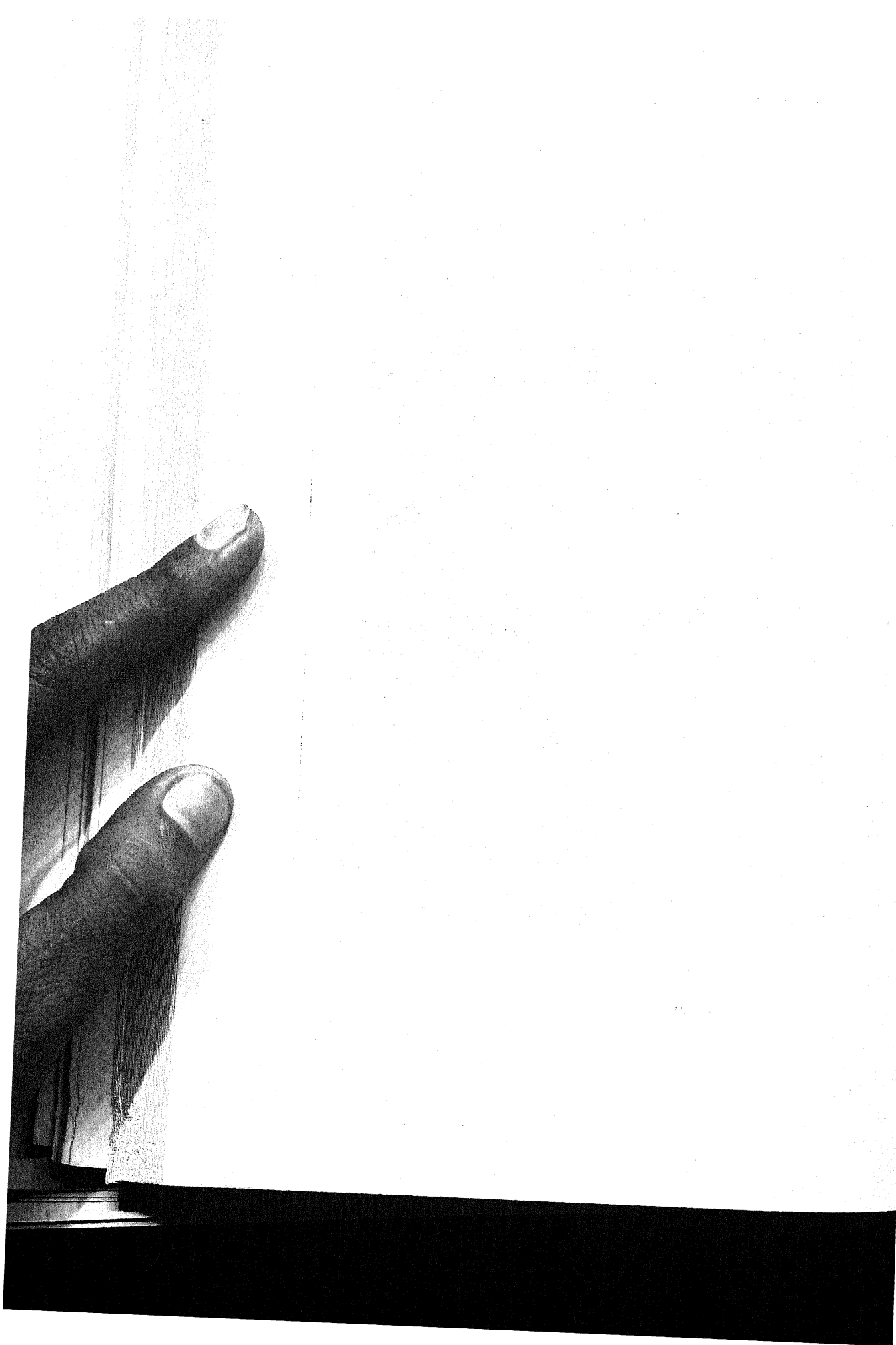


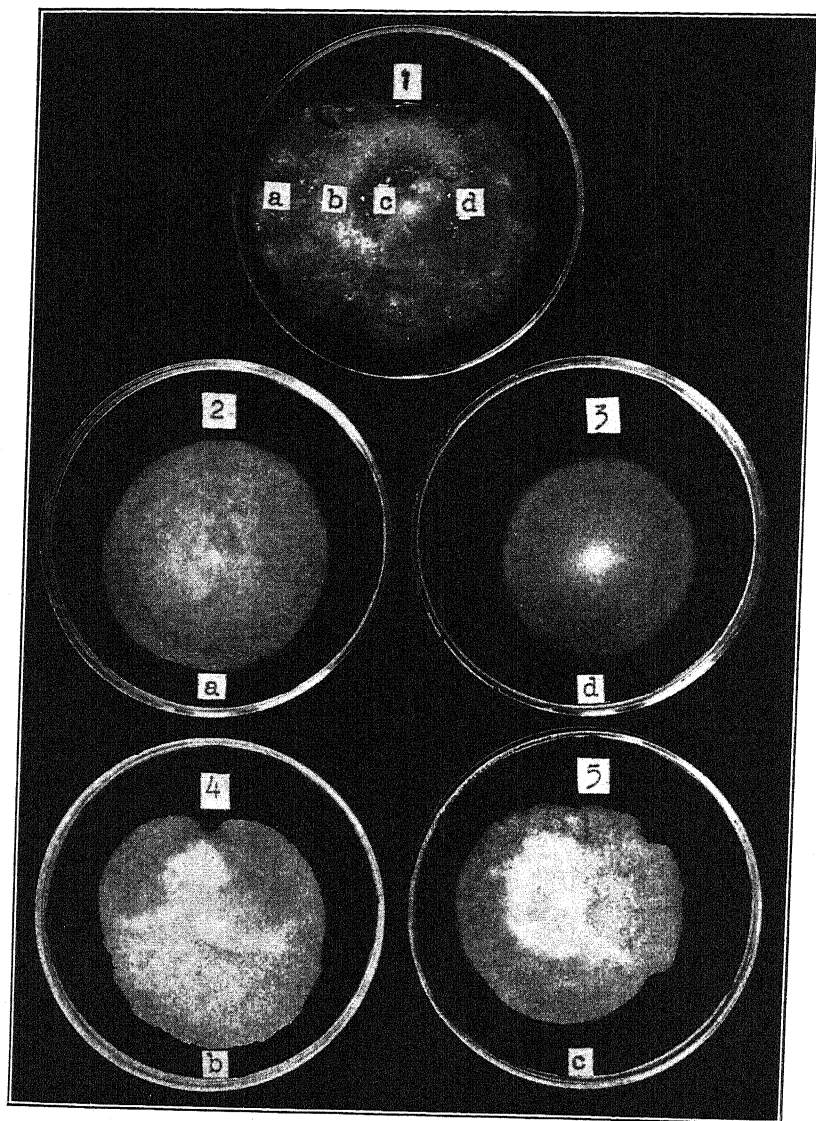
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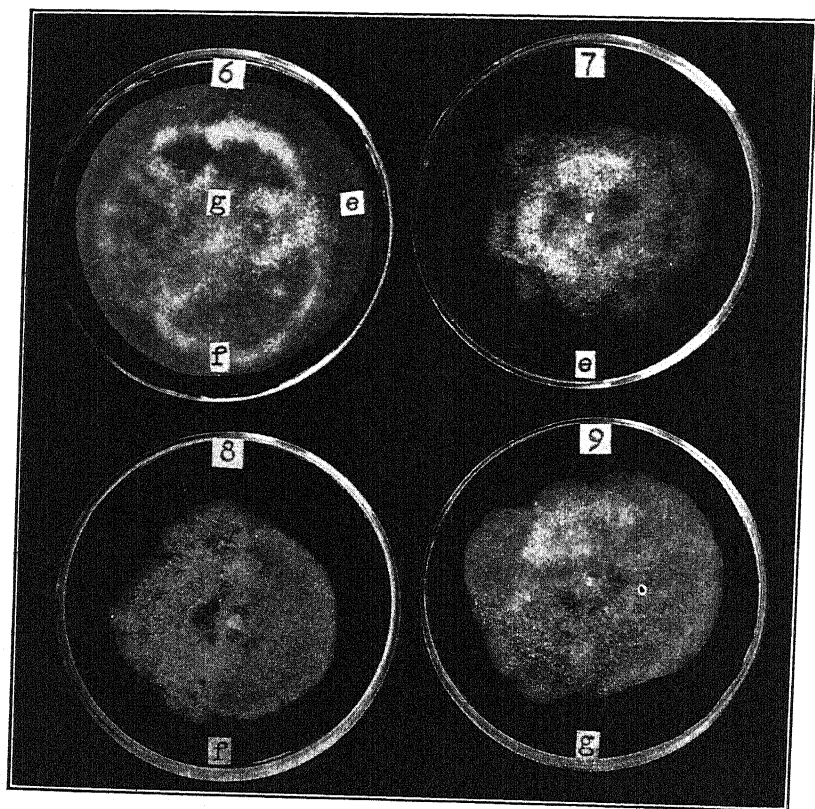


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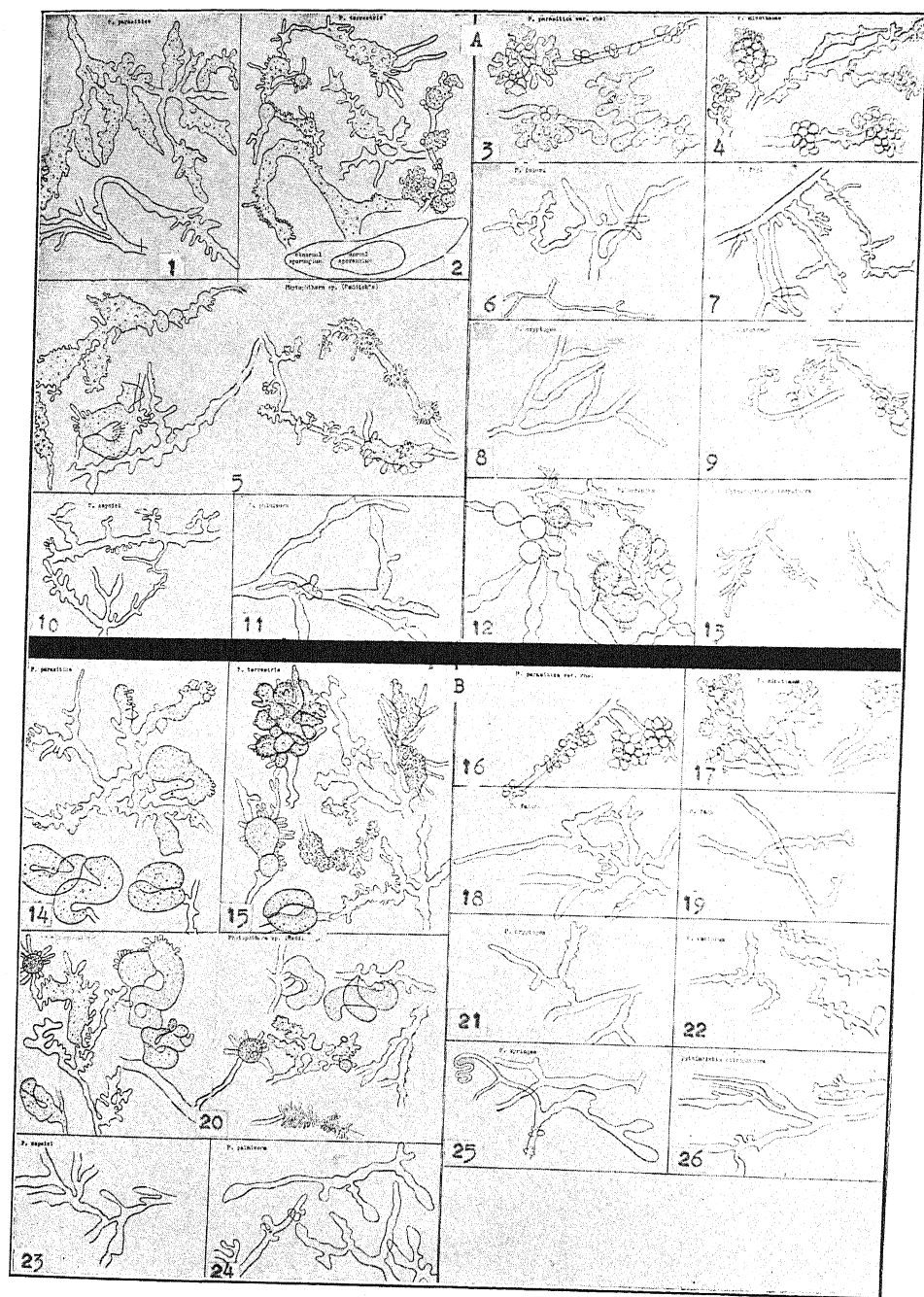




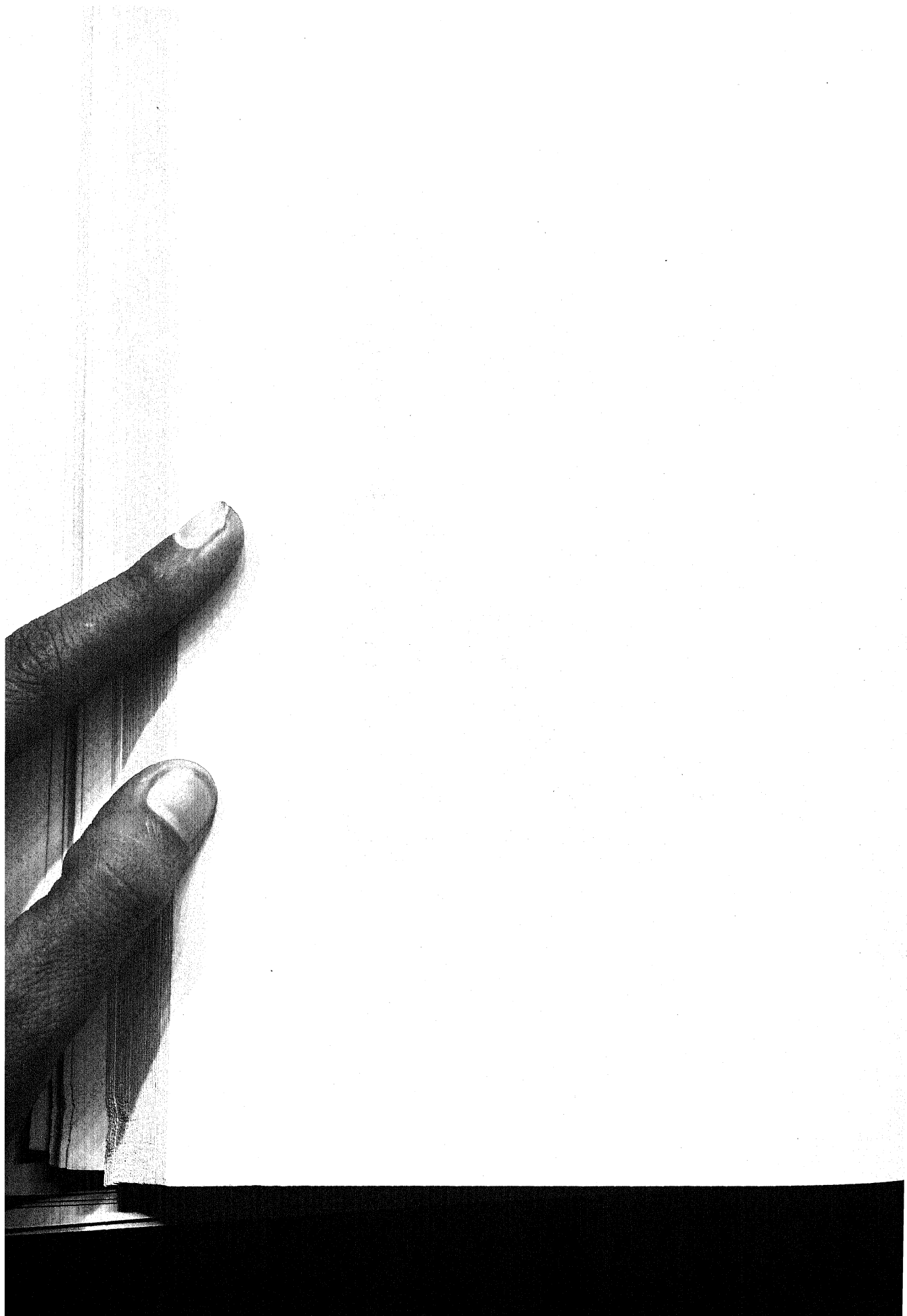
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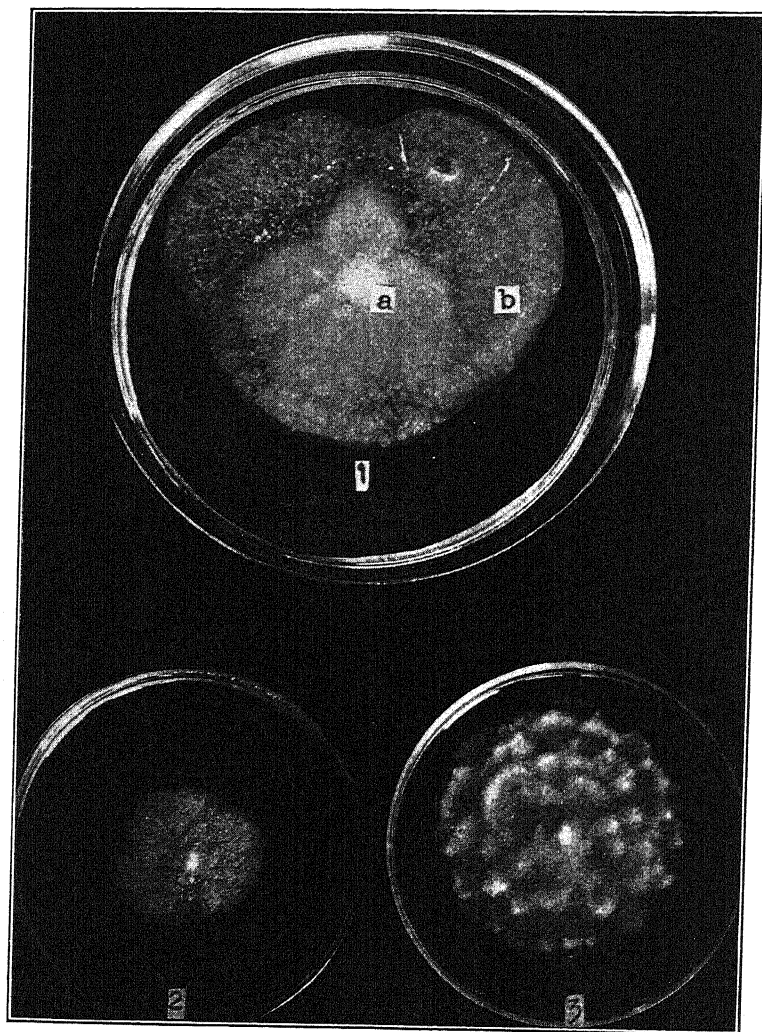






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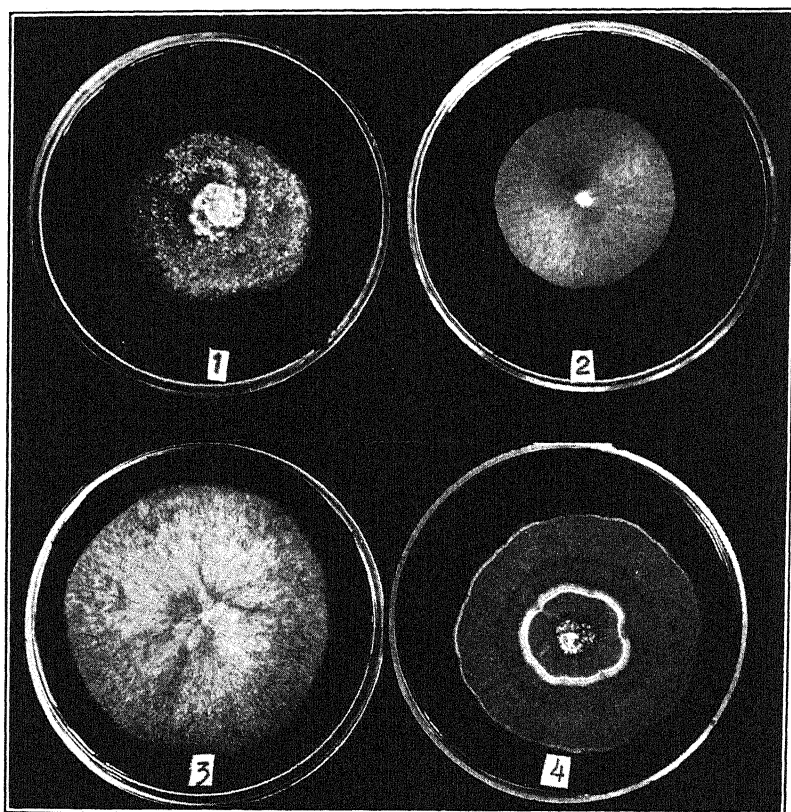




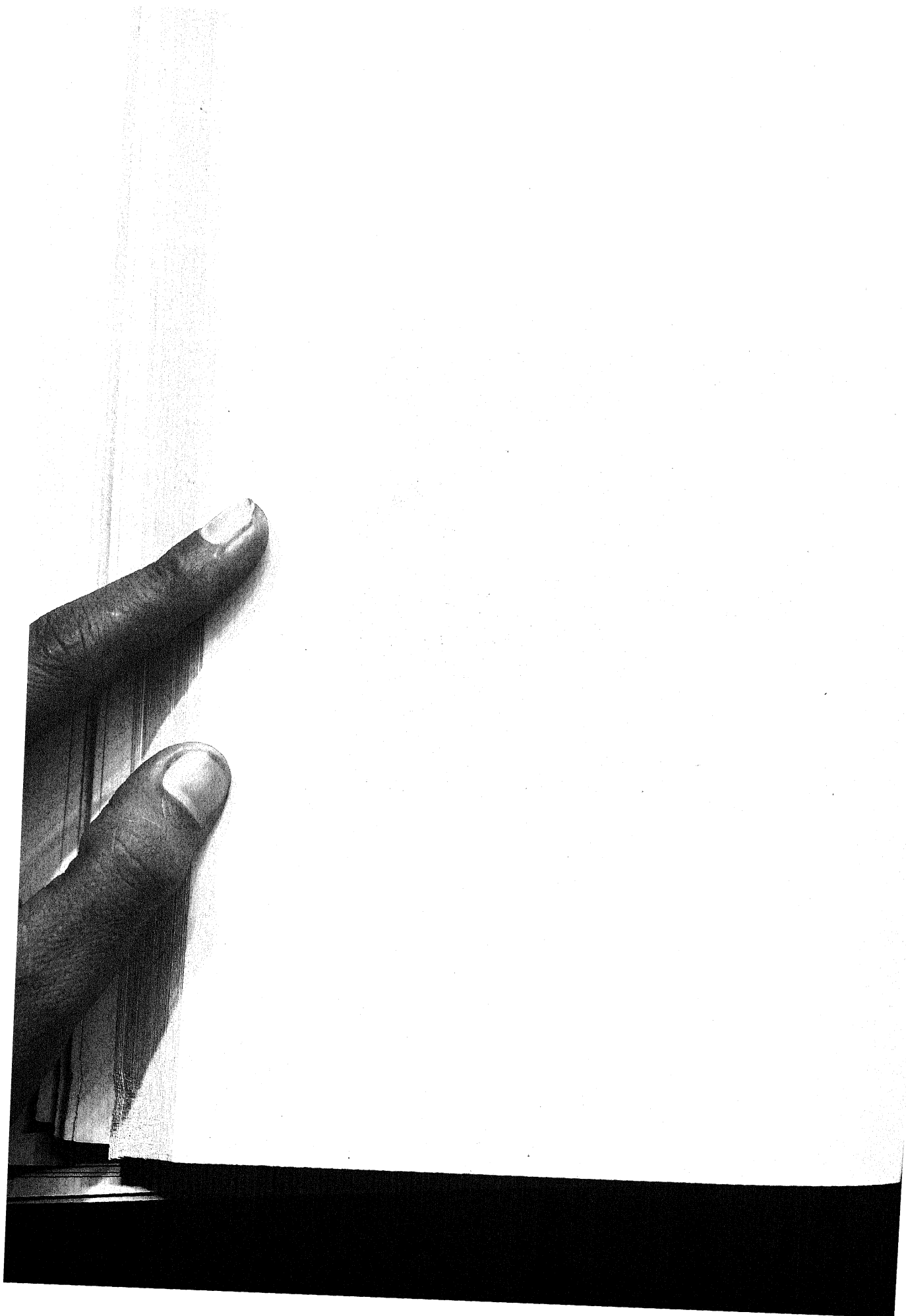
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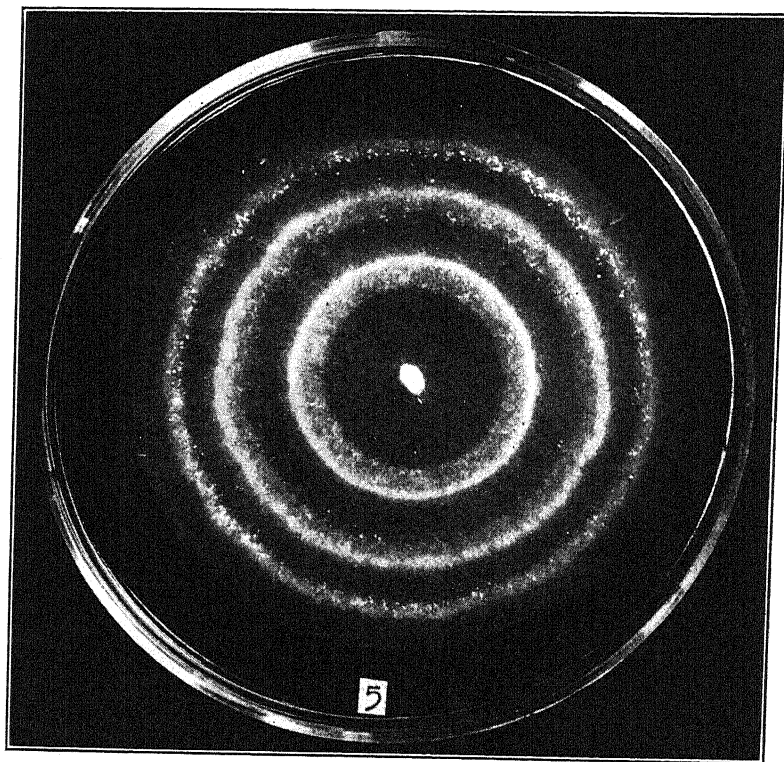




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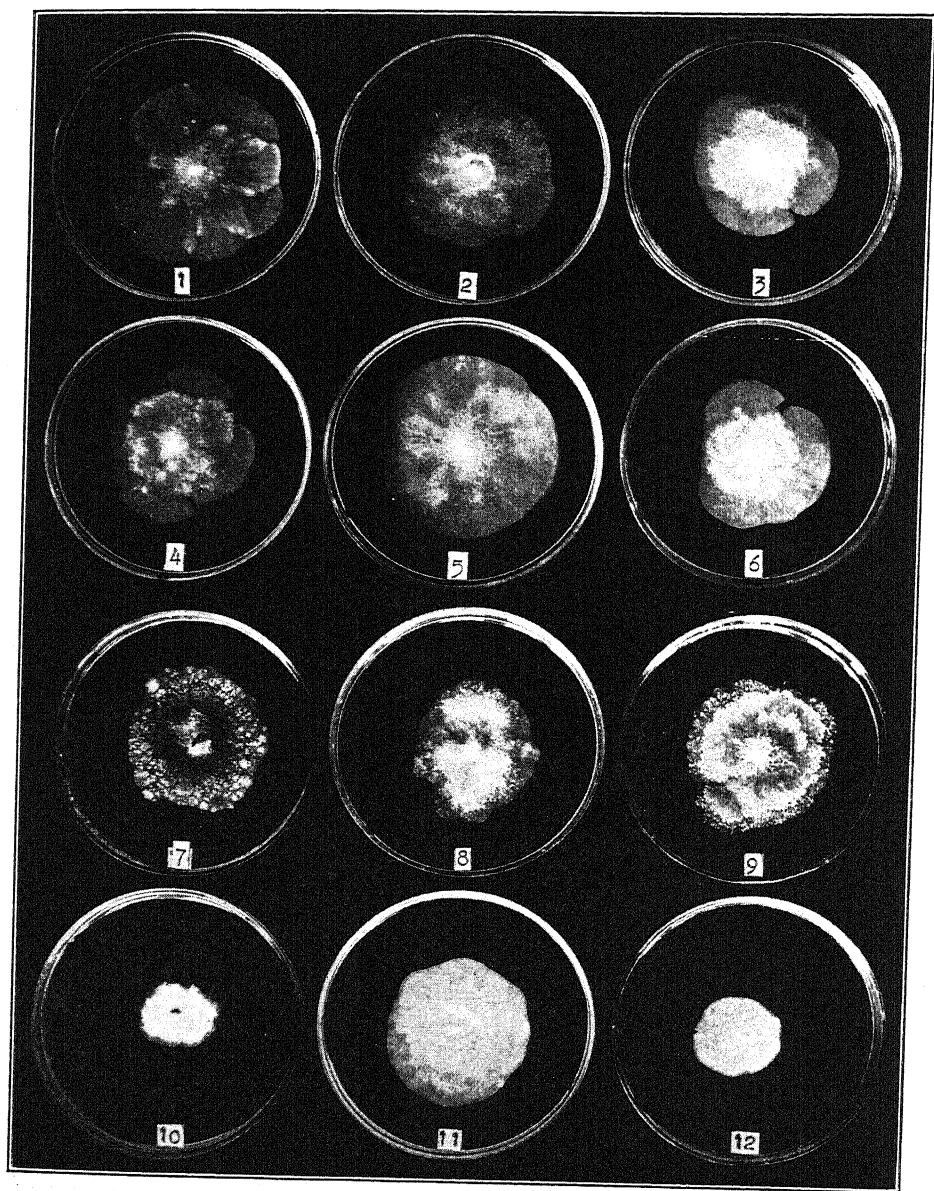




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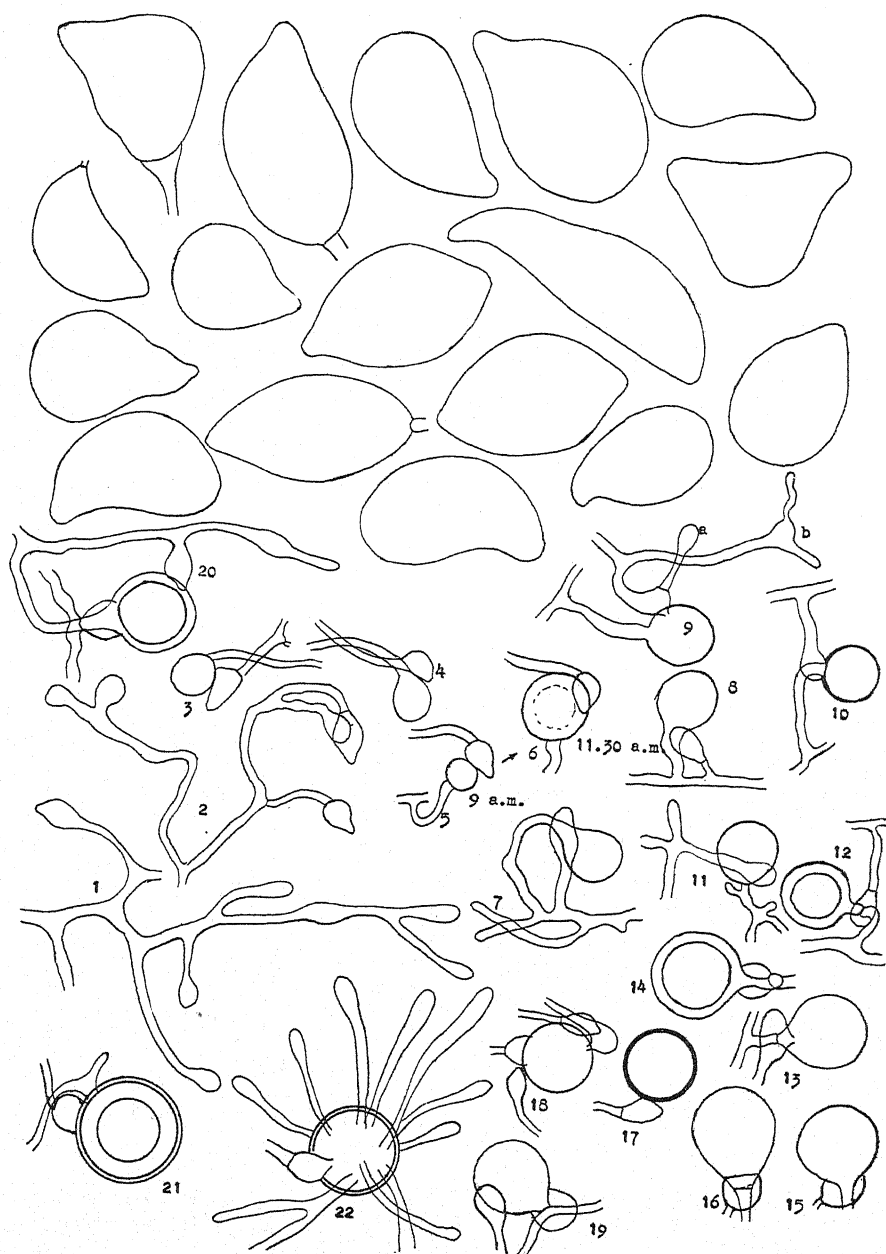






LEONIAN: THE GENUS PHYTOPHTHORA





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## A TABLE TO FACILITATE CORRECTION FOR UNDERCOOLING IN CRYOSCOPIC WORK

J. ARTHUR HARRIS

(Received for publication December 19, 1924)

Since the freezing point of a solution is by definition the temperature at which the solution and crystals of pure solvent are in equilibrium, it is necessary in any careful cryoscopic work to correct the observed temperature at equilibrium,  $\Delta'$  as read from the thermometer, for the concentration of the solution by the separation of the solid phase of the solvent to obtain the true freezing-point lowering of the original solution.

Jones<sup>1</sup> many years ago pointed out that the concentration of the solution by the separation of crystals of solvent may be expressed by

$$f = \frac{su}{l},$$

where  $f$  is the amount by which the solution will be concentrated,  $s$  the specific heat of the solvent,  $u$  the degrees of undercooling observed, and  $l$  the latent heat of fusion of unit weight of the solvent.

Thus in terms of freezing-point lowering and undercooling only,

$$\Delta = \Delta' - \frac{su\Delta'}{l}$$

or

$$\Delta = \Delta'(1 - su/l).$$

The tabling of  $1 - su/l$  for the range of  $u$  to be encountered in practice obviously greatly facilitates the carrying out of correction for undercooling. I have used manuscript tables of this kind for the past several years with great advantage. Unfortunately they are quite too bulky for publication in the ordinary form.

In the case of water, 1 and 80 may be taken as the values of  $s$  and  $l$  sufficiently exact for practical purposes, and we may write<sup>2</sup>

$$\Delta = \Delta' - 0.0125u\Delta' = \Delta'(1 - 0.0125u).$$

Jones, H. C. Zeitschr. Physik. Chem. 12: 624. 1893.

<sup>2</sup> Harris, J. A., and Gortner, R. A. Amer. Jour. Bot. 1: 75-78. 1914.

[The Journal for July (12: 359-498) was issued Aug. 5, 1925.]

Advantage may be taken of one of the properties of the index  $1/80$  to condense the table to printable limits by splitting both the undercooling and the tabled corrective factors for  $\Delta'$  into two sets of entries. The results are given in table I for a range of undercooling of 0.01 to 7.99 degrees.

TABLE I

Degrees Undercooling				Final Three Places of Factor							
6	4	2	0	875	750	625	500	375	250	125	000
First Three Places of Factor				Hundredths of Degrees Undercooling							
.925	.950	.975	1.000	—	—	—	—	—	—	—	.00
.924	.949	.974	.999	.01	.02	.03	.04	.05	.06	.07	.08
.923	.948	.973	.998	.09	.10	.11	.12	.13	.14	.15	.16
.922	.947	.972	.997	.17	.18	.19	.20	.21	.22	.23	.24
.921	.946	.971	.996	.25	.26	.27	.28	.29	.30	.31	.32
.920	.945	.970	.995	.33	.34	.35	.36	.37	.38	.39	.40
.919	.944	.969	.994	.41	.42	.43	.44	.45	.46	.47	.48
.918	.943	.968	.993	.49	.50	.51	.52	.53	.54	.55	.56
.917	.942	.967	.992	.57	.58	.59	.60	.61	.62	.63	.64
.916	.941	.966	.991	.65	.66	.67	.68	.69	.70	.71	.72
.915	.940	.965	.990	.73	.74	.75	.76	.77	.78	.79	.80
.914	.939	.964	.989	.81	.82	.83	.84	.85	.86	.87	.88
.913	.938	.963	.988	.89	.90	.91	.92	.93	.94	.95	.96
.912	.937	.962	.987	.97	.98	.99	—	—	—	—	—

Degrees Undercooling				Final Three Places of Factor							
7	5	3	1	875	750	625	500	375	250	125	000
First Three Places of Factor				Hundredths of Degrees Undercooling							
.912	.937	.962	.987	—	—	—	.00	.01	.02	.03	.04
.911	.936	.961	.986	.05	.06	.07	.08	.09	.10	.11	.12
.910	.935	.960	.985	.13	.14	.15	.16	.17	.18	.19	.20
.909	.934	.959	.984	.21	.22	.23	.24	.25	.26	.27	.28
.908	.933	.958	.983	.29	.30	.31	.32	.33	.34	.35	.36
.907	.932	.957	.982	.37	.38	.39	.40	.41	.42	.43	.44
.906	.931	.956	.981	.45	.46	.47	.48	.49	.50	.51	.52
.905	.930	.955	.980	.53	.54	.55	.56	.57	.58	.59	.60
.904	.929	.954	.979	.61	.62	.63	.64	.65	.66	.67	.68
.903	.928	.953	.978	.69	.70	.71	.72	.73	.74	.75	.76
.902	.927	.952	.977	.77	.78	.79	.80	.81	.82	.83	.84
.901	.926	.951	.976	.85	.86	.87	.88	.89	.90	.91	.92
.900	.925	.950	.975	.93	.94	.95	.96	.97	.98	.99	—

Degrees of undercooling are given for even (0, 2, 4, 6) and odd (1, 3, 5, 7) degrees at the heads of the columns at the left. To these are to be appended the hundredths of degrees given in the body of the table. These two entries taken together give the value of  $u$ . It is idle to consider this to more than the second decimal place.

The first three places of the factor are given *in the columns beneath the*

degrees, the last three places *at the head* of the columns of hundredths of degrees. The facility with which the table may be used is illustrated by the following examples.

$\Delta'$	$u$	$(1 - su/l)$	$\Delta = \Delta'(1 - su/l)$
5.883	0.30	.996250	5.861
2.128	2.99	.962625	2.048
1.315	4.47	.944125	1.242
1.700	3.00	.962500	1.636
0.388	2.34	.970750	0.377
1.971	6.03	.924625	1.822

DEPARTMENT OF BOTANY,  
UNIVERSITY OF MINNESOTA

## INVESTIGATIONS ON THE ROOT HABITS OF PLANTS<sup>1</sup>

J. E. WEAVER

Ten years ago, while studying the vegetation of semi-arid eastern Washington, interest in root studies was aroused because of the extensive root systems of the native species. *Balsamorhiza sagittata*, the first plant excavated, is representative. The strong tap root reaches a depth of six feet, and very numerous branches run laterally to distances of about two feet. Further investigations in several other states showed that extensive root systems are the rule among grassland species. *Andropogon hallii*, with exceedingly well branched fibrous roots seven feet deep, is representative of many of the dominants. About ninety percent of the more important species are rooted well below the two-foot level, and not a few, such as *Liatris punctata*, extend to depths of fifteen to twenty-two feet.

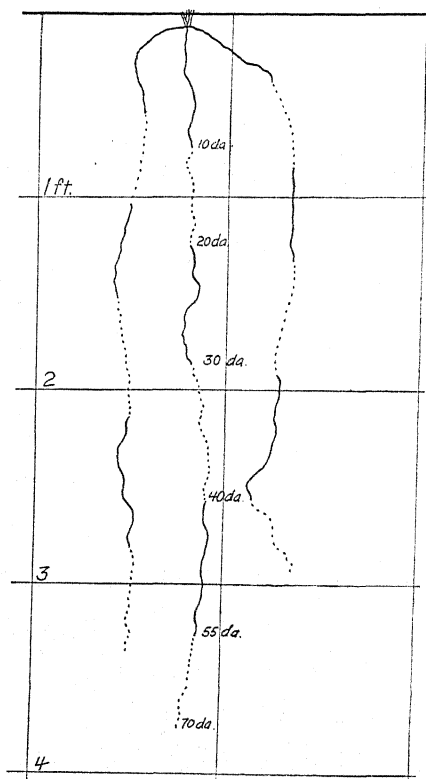
The great extent of roots in relation to above-ground parts is often very striking. *Gleditsia triacanthos*, thirteen weeks after seed-germination, although reaching a height of only nine inches, produced a remarkably widespread root system that extended well into the fourth foot of soil. The roots of *Solidago oreophila*, a small species only about a foot high, spread about two feet on all sides of the plant while some penetrate downward to a distance of two and one half feet. Nor is the great extent of roots in comparison to tops confined to native species. Maize (*Zea mays indentata*) has a wonderfully developed root system which occupies rather thoroughly over two hundred cubic feet of soil. The root system of the sugar beet (*Beta vulgaris*) is likewise very extensive, branching widely and extending downward to a depth of five or six feet.

The rapidity of root growth is quite as remarkable as root extent. *Spartina cynosuroides* reaches a depth of over four feet at the age of eleven weeks. This growth rate of over half an inch a day is not unusual among our native grasses. The widely spreading roots of potatoes, when they begin their vertical descent, elongate at the rate of an inch a day for a period of two or more weeks. When the main vertical roots of corn begin to develop, they sometimes penetrate downward at the remarkable rate of 2 to 2.5 inches per day during a period of 3 or 4 weeks, sometimes reaching depths of more than eight feet. The rate of growth of the primary root system of winter wheat during 10- or 15-day intervals is of interest (text fig. 1). An average growth rate of over half an inch per day is maintained for a period of 70 days. Of course, during this time abundant laterals and also roots of the secondary system are developing.

<sup>1</sup> Invitation paper read at the joint meeting of Section G, American Association for the Advancement of Science, the Botanical Society of America, the American Phytopathological Society, and the American Society of Plant Physiologists at Washington, December 30, 1924.



That large amounts of both water and nutrients are absorbed by these deeply penetrating roots has been repeatedly demonstrated. By means of wax seals placed at various depths in filling large containers, soil layers of known water and nutrient content have been effectually isolated from the layers above and below. Through these seals roots penetrate readily and

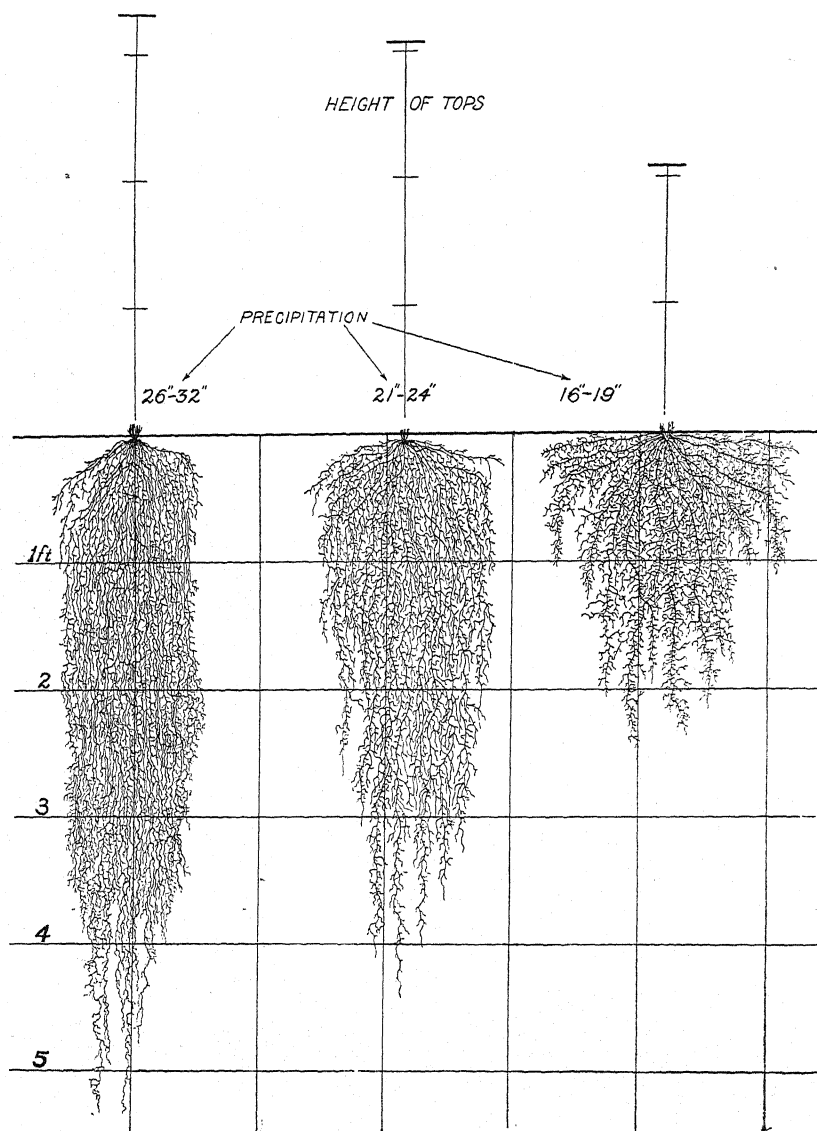


TEXT FIG. 1. Diagram from field measurements showing the rate of growth of winter wheat during 10- and 15-day intervals.

develop normally. By this means it has been ascertained, for example, that barley absorbed 19 percent of water, based on the dry weight of the soil, from the first foot, 16 percent from the second, and 13 percent from the third, while 11 percent was absorbed from the fourth foot of soil. Barley, like maize, absorbed large quantities of nitrates from the deeper soil. Maize removed 203 parts per million of nitrates from the third foot, 140 from the fourth, and 118 from the fifth.

Variations in root habit under different climatic conditions are often very pronounced. Continued examination of the smaller cereals in fertile silt-loam soils under a wide range of precipitation and soil moisture shows that the root habit varies widely (text fig. 2). Under 26 to 32 inches of pre-

cipitation, such as occurs in eastern Kansas and Nebraska, the tops of winter wheat are tall and the roots deep, but the lateral spread is relatively small. But in western Kansas and eastern Colorado, where 16 to 19 inches of precipitation wet the soil to only 2 to 2.5 feet, the tops are short, and the roots are shallow but very widely spreading and much more profusely branched. Root habit under an intermediate precipitation of 21-24 inches falls between



TEXT FIG. 2. Diagram showing the growth of root and shoot of winter wheat in fertile silt-loam soil under different climates.

these extreme types, but is correlated with a medium development of shoot. Two-year-old alfalfa, in fairly moist, deep soil, penetrates with little branching of the tap root to depths of from 10 to 12 feet. But on the Great Plains, where a subsoil lacking available water prevents downward penetration, so many profusely branched, widely spreading laterals are produced that one would hardly recognize the roots as those of alfalfa.

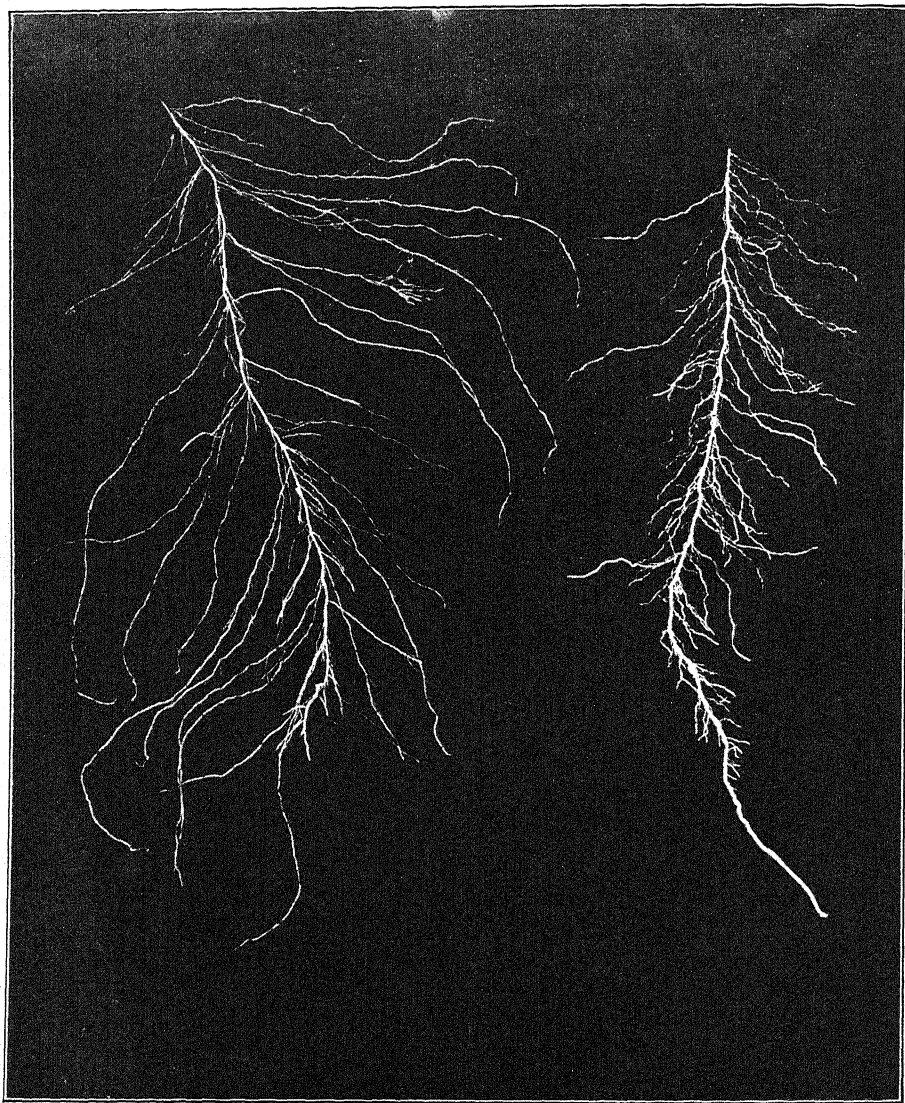
Variations under local environments are also very great. The effects of the addition of nitrates, for example, in retarding root-elongation and promoting branching are well known. Sugar beets of the same age and grown in similar soil show marked differences. One plot was manured and irrigated, whereas in the other the soil was very dry and the deeper subsoil was almost without available water. Differences in root habit were shown in depth of penetration, and in number, length, and position of lateral branches. In the dry land the beets had their normally deeply penetrating tap roots limited because of dry subsoil in their early development to only one half the depth in the moist soil, and throughout the season they were 1 to 1.5 feet shorter. Branching was more profuse and sublaterals were more abundant than in fully irrigated soil.

Root-stratification due to soil texture is not infrequent. In one experimental plot, layers of clay occurred in the fine-sandy loam at depths of 16 inches and 3.5 feet respectively. Into these layers of greater nutrient content densely branched roots of sugar beet ran horizontally in great profusion, contrasting in a striking manner with the more poorly branched parts of the root system above and below.

Six-weeks-old maize grown in a fine-sandy loam with only 4 to 6 percent of available water had a very different root habit from that grown in an otherwise similar but manured soil with a nearly optimum water content. In the watered plot the roots were very near the surface, being almost entirely in the first 8 inches of soil. But in the unirrigated plot, where the surface soil was very dry and not so rich in nutrients, the roots penetrated more deeply and the branches were more than twice as numerous. Moreover, these laterals were double the length of those in the rich, moist soil and were clothed with approximately twice as many fine sublaterals.

Pronounced differences in root-development in the same field may also be induced by competition. Spring wheat grown at the normal field rate of planting was more deeply rooted when mature than wheat planted four times as thickly. The working levels were 39 and 35 inches respectively. But in proportion to tops, plants of the thicker stand had much more extensive root systems. Competition for water and nutrients had reduced the supply to such a degree that, although in adjoining plots, the thickly planted crop was growing in a much drier and less fertile soil. Root habits of sunflowers planted respectively 2, 8, and 32 inches apart were very different. The working levels of the tap roots were, in the preceding sequence, 5, 6.5, and 8 feet. Those of the numerous laterals were 12, 37, and 48 inches.

Moreover, the lateral spread varied from 10 inches in the thickest plantings to 42 inches in the thinnest. Although the water content was about the same in all three plots throughout the season and the nitrates were constantly reduced to a minimum, yet the more widely spaced plants had more of both these essentials. Likewise, they had more light for food-manufacture, and the better top development afforded an abundance of food for root-building.



TEXT FIG. 3. Portions of the primary root system of maize; (left) from wet loess and (right) from moist sandy soil.

However, when based either on leaf expanse or on dry weight of tops, the nitrogen-starved, dwarfed plants in the crowded environment had relatively a more extensive root-development.

Considerable progress has been made in a study of the relation of absorbing area to transpiring surface. Methods have been devised by means of which it has been found possible to secure root systems several feet in extent quite in their entirety from the soil in which they grew. Indeed, microscopic examination showed that the root tips of even the most delicate laterals were wholly intact. The large amount of work involved in securing and accurately measuring the surface of even a single root system may be partly appreciated when we find that a plant of maize only in the eighth-leaf stage has from 8,000 to 10,000 laterals arising from the 15 to 23 main roots.

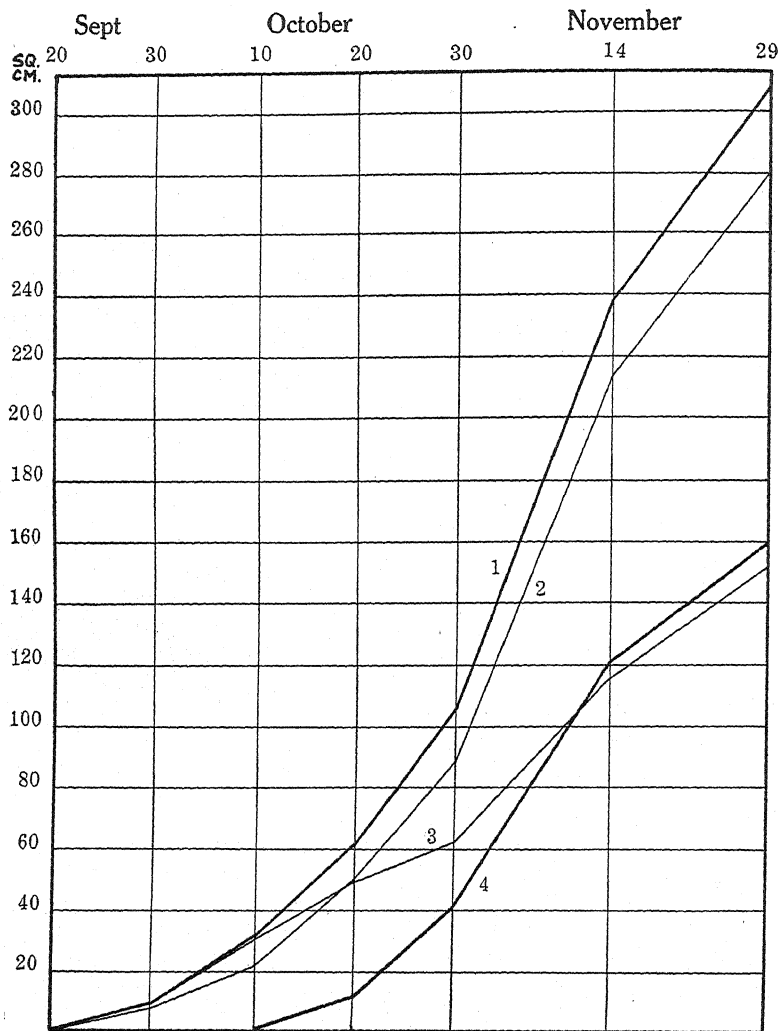
Nebraska White Prize corn of the  $F_1$  generation from two pure-line parental strains, and consequently of similar hereditary constitution, was grown for 5 weeks in fertile loess soil at water contents of 9 and 19 percent respectively above the hygroscopic coefficient. In the wet soil the area of the tops (including the stem and counting both surfaces of the leaves) was 82 percent of that of the roots. But in the drier soil the tops had only 46 percent as great an area as the roots. In other words, the absorbing area of the roots (exclusive of root hairs—which covered the entire system but which, of course, were not measured) was 1.2 times as great as the area of tops in the wet soil and 2.2 times as great in the drier soil. The total length of the main roots in the two cases was about the same, as was also their diameter. In neither case did the main roots make up more than 11 percent of the total absorbing area. In the drier soil 75 percent of the area was furnished by the primary laterals and the remaining 14 percent by branches from these. But in the wet soil the primary branches furnished only 38 percent of the root area. It seemed as though the plant had blocked out a root system quite inadequate to meet the heavy demands for absorption made by the vigorous top, and the remaining 51 percent of the area was furnished by an excellent development of secondary and tertiary branches.

The large area of a single root is often impressive. Text figure 3 (left) shows about two thirds of the primary root of corn from wet soil. This portion had an area of 132 square centimeters, or only slightly less than one third of the area of this page. In fact, the three roots of the primary system constituted 49 percent of the entire absorbing area.

Plants in loess soil with only 2 to 3 percent of water in excess of the hygroscopic coefficient had, in proportion to the length of the main roots, about one third more laterals than those in a similar soil of medium water content. Moreover, the absorbing area, in comparison to tops, was greater.

Corn grown in sandy soil, with an optimum water supply but a higher nitrate content than the loess, had an intermediate root area (text fig. 3, right). It was 1.3 times as great as the area of the above-ground parts.

Aside from the 10 percent total absorbing area furnished by its main roots, the remainder was divided almost equally between primary and secondary laterals, no tertiary branches occurring.



TEXT FIG. 4. Graphs showing: 1, the absorbing area of winter wheat (exclusive of root hairs); 2, the photosynthetic and transpiring area; 3, the absorbing area of the primary root system; 4, the absorbing area of the secondary root system.

The relation between the area of roots and the area of tops of a variety of Turkey Red winter wheat grown under field conditions has been determined at intervals of 10 to 15 days (text fig. 4). Graph 1 shows the root area at the end of the several periods, graph 2 the area of the above-ground parts at the same times. The parallelism of the two graphs is very striking, as is also

the continuously greater root-absorbing surface compared to the photosynthetic and transpiring area. Root area exceeded area of shoot by 10 to 35 percent. Graph 3 shows the proportion of the entire absorbing surface (exclusive of root hairs) furnished by the primary root system; and graph 4 the part afforded by the secondary root system. The rapidity of the development of the latter is marked, exceeding at the end of 35 days of its growth the area of the primary root system. Another feature was the persistence of functioning root hairs. Not until 7 weeks after planting did the root hairs begin to slough off from even the oldest part of the primary roots, and 3 weeks later all but one third of one percent of the entire root area was clothed with functioning root hairs. Of course in very dry soils these are shorter-lived and many of the branch roots from which they arise may also die.

Adaptation of crop plants to new environments whether by selection or by breeding, like so many other problems in plant-production, warrants careful study of root relations.

UNIVERSITY OF NEBRASKA

## SOIL NUTRIENTS IN RELATION TO VEGETATION AND REPRODUCTION <sup>1</sup>

E. J. KRAUS

Investigations dealing with soil nutrients have been of several types. Among them may be mentioned those designed to test which elements are to be classed as essential; those which have attempted to determine the combinations of these elements suitable or necessary for plants in general, or for specific plants; those which have endeavored to discover the effect of variations of the relative concentrations of these compounds upon plant development; those which have dealt with the variations in the rates at which the various compounds, or portions of them, are absorbed by the plant during successive stages in its development, and, somewhat more recently, under variations in temperature and a few other external factors; those which have been concerned with the assumed effect of the reaction of the nutrient medium upon absorption; and others which have had to do with various special or individual problems. Out of this work have developed various theories concerning the power and methods of plant "feeding," enormous numbers of analyses of soils, extensive and intensive fertilizer experiments. As yet, however, the rôle which any one of the so-called essential elements plays in plant-development is exceedingly imperfectly known.

That progress of knowledge should be slow is to be expected, of course, when one considers that most of the essential elements probably enter into the make-up of from several to scores of compounds, which in the final analysis constitute and are the plant. Nor can much advance in the study of plant nutrition be expected merely from applications of various compounds to plants and subsequent attempts at recovery of these compounds, or the elements of which they are made up, on the basis of ash analyses or the determination of the total quantity of such elements in plant tissues. Such experiments have yielded and will continue to yield results of varying degrees of value in local tests and fertilizer trials, but often they do not give even the slightest hint as to the part an element has played in the production of the observed result. In like manner, attempts to explain the intake of nutrients mainly on the basis of the action of a plasma membrane as a regulator of osmotic phenomena prove less and less satisfying. More recently, studies in colloid phenomena have seemed to offer greater possi-

<sup>1</sup> Invitation paper read at the joint meeting of Section G, American Association for the Advancement of Science, the Botanical Society of America, the American Phytopathological Society, and the American Society of Plant Physiologists at Washington, December 30, 1924.



bilities in affording an explanation in keeping with observed facts. The work dealing with the electrical behavior and the amphoteric character of proteins, universally occurring and constituting a considerable portion of all living matter, suggests that many more investigations of similar sort will yield results of the greatest value. As yet, however, the hypotheses trying to account for nutrient intake as an electrical phenomenon are so very generalized that they can be regarded only as suggestions.

If it may be assumed that theories relating to the behavior of colloids are to be of value in a study of the problem of nutrient intake and of metabolism, then something of the kinds and nature of colloids within the plants serving as experimental material must be known, and conditions for their generation, maintenance, and destruction must be studied. Such a study would certainly involve, likewise, an estimation and determination of those non-colloidal substances which serve as the building stones for the synthesis of proteins and other colloids. Likewise, the substances into which such materials are re-resolved by means of hydrolysis or by some other method must be considered. It is essential to know what relation one class of substances may bear to another, and to correlate these relationships as they may exist within the plant with the behavior and appearance of the plants under investigation. Something of this sort has been done, though scarcely a real beginning has been made. More perhaps has been done with the intake and working over of the compounds of nitrogen than with those of any other one of the elements derived from the soil. It may not be amiss, therefore, to review some of the types of attack which have a bearing upon the relationships suggested above, even if such a review be fragmentary.

What forms of nitrogen various green plants take in from a surrounding nutrient medium has been generally determined, but the factors influencing or governing the method and rate of such intake are far from being well known. Conditions of environment other than the concentration of nitrogenous and other salts in the nutrient solution play a very important part in the intake of nitrogenous salts. Harvey and True (6) some years ago pointed out that a concentration of magnesium nitrate which proved toxic to seedlings grown in the dark was not harmful when such seedlings were exposed to light. This result was accounted for by the suggestion that the magnesium was combined in the chlorophyll molecule in the latter instance and that its toxicity was thereby reduced. It is quite as possible also that the effects observed were related to changes in the nitrate ion, a very possible change being that, in association with carbohydrates, it was built into some more complex organic compound. That this might be possible is even more strongly suggested by the work of Miss Reid (8), who found that cuttings of tomato plants containing a large reserve of carbohydrates subsequently utilized more of this reserve in growth, in darkness, when supplied with a nutrient solution containing nitrates. Miss Eckerson (3) reports that, upon addition of nitrates to juice expressed from carbohydrate-high tomato plants

and adjusted to pH 7.6, there was a more rapid reduction of the nitrates than in the case of the juice extracted from nitrogen-high plants. In some of my own experiments it was found that tomato plants, high in such carbohydrates as sugar, starch, and hemicellulose, removed nitrate nitrogen from a nutrient solution more than five times as rapidly as plants of the same weight but relatively low in such carbohydrates, and that the former plants grew far more rapidly than the latter until such carbohydrate reserves had been utilized.

As will be brought out more particularly later, it is essential to consider not only the composition of the plant as a whole, as a determiner of the course of utilization of the materials available to it, but such composition must be correlated with the state and the development of its separate parts. There are scores of available records which show that the nutrient elements are not removed from the nutrient medium at the same rate or in the same proportions at the various stages in the development or maturity of the individual, and likewise, in turn, that the individual is markedly influenced in its development by variations in the time at which the nutrients are supplied to it. Furthermore, one can not be content with considering composition alone. A plant bearing fruits or seeds will develop very differently from one without them, or, if these are not present and their formation or differentiation is initiated, the subsequent vegetative behavior of the plant will be vastly modified.

The relation of the nitrogenous content of green plants to the production of vegetative parts, flowers, and fruits has been pointed out by a number of investigators. Likewise, the carbohydrate content of plants has been studied and estimated from the same point of view. It is natural, perhaps, that from such studies attempts should have been made to show a quantitative correlation between the nitrogen and carbohydrate content of a plant on the one hand and its condition, whether vegetative or fruitful, on the other. These attempts resulted in the formulation, first perhaps by Fischer (4), of a carbohydrate-nitrogen ratio the value of which would vary directly with the condition of the plant, being relatively high for the condition of flowering and relatively low for the vegetative condition. That the relation of the nitrogenous content of plants to their carbohydrate content varies fairly consistently in direct relation to the type of growth being made by a plant and to its general condition, there seems little room for doubt; but that it is possible to compute definite ratios which would mathematically represent such conditions is most decidedly open to question. When one considers the varied rôle played by carbohydrates, and the many different forms of nitrogen found within the plant, it is quite unlikely that any mathematical ratio between total quantities of nitrogen and carbohydrates can be established as causative of a limited set of plant functions. It is remarkable that some of the ratios which have been calculated fit as well as they do. It still remains, therefore, to determine whether there may be some specific

form or forms of nitrogen associated with certain peculiarities of plant behavior, and the relation of these to the soil nutrients. Information on this point is not wholly lacking, or at least the possibilities of obtaining it seem promising.

Some time ago Kraus and Kraybill (7), in working with the tomato, found, when this plant was subjected to varying amounts of nitrates in the nutrient medium, whether this was soil, stable compost, or quartz sand to which culture solutions were supplied, that a fairly constant relationship could be established between the nitrogen content of the plant and its vegetative and fruiting behavior. Under the conditions of their experiments, nitrogen in the nitrate form was always correlated with the highly vegetative condition. When nitrates were withheld the following sequence of changes occurred: the plants became less and less vegetative; carbohydrates accumulated; fruiting at first increased, then decreased rapidly; nitrates within the plant disappeared. Among other things it was assumed that nitrate nitrogen stands in some direct causal relation to the highly vegetative state and that the non-vegetative state results from its lack, even though opportunity for carbohydrate synthesis is provided. On the other hand, it seemed wholly possible that the nitrate nitrogen might very possibly represent little more than a transitory form of nitrogen, or an accumulated, non-metabolized reserve, and that the really effective forms of nitrogen to be associated with the several types of condition observed might be quite different in nature. This latter view seemed strengthened by the fact that many investigators have found it exceedingly difficult to detect nitrates in many woody plants, though such plants may be highly vegetative. Likewise, Woo (9) found that, in *Amaranthus*, relatively large quantities of nitrates were present in fruitful plants which were not highly vegetative.

Except in the case of seeds, some bulbs, tubers, and storage roots, until recently the principal method employed to vary the vegetative and reproductive behavior of plants and to alter the types and quantities of nitrogenous compounds within them was to vary the nitrogenous compounds in the nutrient medium. To be sure, variations in temperature induced marked changes in growth, especially when plants were liberally supplied with nitrogen, but if this supply was restricted the changes were not great. Recently, however, there have been demonstrated two possible methods by which pronounced changes in vegetative and reproductive behavior can be induced without alteration of the nutrient medium in which the plants are growing, whether this medium contains nitrogen or not. The first of these is a direct result of the comprehensive experiments on photoperiodism by Garner and Allard (5). On the basis of their suggestions it is possible to make non-vegetative plants vegetative, and *vice versa*, simply by altering the light conditions under which the plants are grown. Attempts have been made by Mr. G. T. Nightingale to correlate these shifts in behavior with internal composition, in so far as time and technique have permitted. Citation

of but a few cases will suffice. Certain species of plants, when subjected to short periods of light, are feebly vegetative, and fruitful. Such plants are often high both in nitrates and in reserve carbohydrates. If the amount of illumination of such plants is increased, even though the possibility for intake of additional nitrogen is inhibited, vegetativeness is often markedly increased. Such increase is accompanied by a diminution of the nitrate nitrogen within the plant (provided this was present), and by an increase in its amino-nitrogen content—the total content of nitrogen remaining unchanged. As somewhat of a converse of this, some species of plants which are non-vegetative, non-blooming, and non-fruiting, with a shift in the period of illumination become vegetative and frequently bloom. Analyses of such plants have shown that, although these plants may be completely lacking in nitrate nitrogen, yet, like the plants above mentioned, they also may show a marked increase in their content of amino nitrogen; the total content of nitrogen remaining unchanged as in the plants mentioned above. In the one instance the amino nitrogen appears to have been derived from the unmetabolized nitrate nitrogen, in the other from the more complex organic forms; but in both cases it is associated with increased vegetativeness. The association of a relatively high amino-nitrogen content with a vegetative condition is not at all unusual, having been observed many times in developing seedlings and shown to be of common occurrence in plants grown in darkness and under a number of other conditions. In such experiments as have been carried out up to the present time, accompanying the increase in amino nitrogen there is also generally an increase in those nitrogenous fractions (other than nitrates) which may be fairly readily extracted with water from finely ground, fresh tissues. What all these fractions may be chemically, remains to be determined. The point to be emphasized, however, is that in the many experiments conducted, whatever external conditions were imposed, whenever plants were vegetative, amino nitrogen was relatively high, the reserve carbohydrates were relatively low, and nitrate nitrogen was variable. As will be apparent also from what is still to be said, it is entirely possible, therefore, profoundly to shift the nitrogenous content of a plant qualitatively, and, though no change in the total quantity of nitrogen present within the plant has been effected, such qualitative shifting is accompanied by definite, externally measurable responses. Such a reworking of compounds has been demonstrated also for phosphorus and sulfur, and probably is quite general for most of the essential elements as long as the materials of which they form a part are not entirely removed from contact with the living portions of the plant.

One of my associates, Mr. A. E. Murneek, studying the tomato, has worked with a second method by means of which it is possible to manipulate the vegetative behavior of a plant in a manner quite different from that above described but equally effective. He found that, if a plant is subjected to a low plane of nitrogen supply, the presence of a single developing fruit may completely suppress the vegetative activity of the plant. If, however, this

fruit is removed, although no nitrogen is supplied in the nutrient medium, vegetativeness is quickly resumed and may continue for a considerable period—frequently until another fruit is formed. In this way it was easily possible to secure material for chemical analyses, which, like the experiments on photoperiodism, resulted in showing the same relation of increased amino nitrogen accompanying the condition of active vegetativeness, but showing that nitrate nitrogen within the plant is not essential to such growth. It was found that plants growing in a nutrient medium rich in nitrate nitrogen, when the fruits were prevented from forming on them, were highly vegetative and did indeed contain relatively large quantities of nitrates, whereas plants growing under similar conditions, except that the developing fruits were allowed to remain on them, were far less vegetative, much higher in reserve carbohydrates, and the proportion of nitrates was much less, though in some cases these latter were relatively abundant also. The developing fruit exercises a powerful influence in diverting to itself both the nitrogen within a plant and that which is more or less steadily being removed from a nutrient medium. With an abundant supply of nitrogen, plants such as the tomato, which may be vigorously vegetative, blooming, and fruiting at one and the same time, remain in this condition only so long as there is a copious supply of nitrogen in the nutrient medium and ample opportunity for carbohydrate synthesis. Just as soon as the nitrogen is restricted, vegetation decreases, carbohydrates accumulate, and somewhat later fruit-setting ceases and even the differentiation of flowers is stopped. The removal of the fruits from such plants, or the addition of nitrogen to the nutrient medium if nitrogen has been limiting, brings about the reverse of this series of changes. It is worth while here to call to mind the recent determinations of Campbell (1), who found the nitrate-nitrogen content of various weeds highest just before blooming and zero at full maturity.

It should be remembered also that it is possible to inhibit fruit-setting by restricting the opportunities for carbohydrate synthesis, a situation which would appear necessarily to be true when the rôle of carbohydrates in the formation of more complex nitrogen-containing substances, or in various other plant processes, is considered. Though stressed many times previously, it is worth reëmphasizing that, with reference to carbohydrate and nitrogen supply alone, two classes of non-vegetative, non-reproductive plants are possible, the one due to a deficit in carbohydrate synthesis, the other to a deficit in synthesis of nitrogenous compounds.

By means of these several avenues of approach, as well as by examining germinating seeds and seedlings developing both in the light and in the dark, it seems that it will be possible in time to come close to tracing the course of nitrogen within the plant, to discover the forms effective in the cause or maintenance of definite states or conditions, and also to determine the influence these substances may have on the absorption of additional increments of nitrogen in its various forms from the nutrient medium. It seems more than likely that nitrates within the plant represent mainly a nitrogen

reserve, but by their presence they would also exercise a marked influence on the rate of absorption of nitrates from the nutrient medium.

The comparatively few records which are available relative to sulfur and phosphorus within the plant indicate that relatively large quantities of sulfates and phosphates may also exist or accumulate as reserves within the plant, and that these may be metabolized when a deficit of these substances is created in the nutrient medium. Like nitrogen, sulfur and phosphorus may enter largely into organic combinations. The study of the interrelationships of these elements or of their simpler compounds with the carbohydrates, fats, proteins, and other organic substances contained in plants has not been wholly neglected, but a much more intimate study of the problem will certainly be worth while.

It seems unfortunate that detailed studies such as Miss Eckerson (2) began in attempting to trace the course of the nutrient elements in the wheat plant can not be greatly extended. With what knowledge has been gained from the study of the effects of various nutrient solutions, hydrogen-ion concentration, and the chemical and physical character of soils, and with the knowledge that will continue to be contributed from such sources, it seems that the work already in progress at many places dealing with the course of the nutrients within the plant could with greatest profit be greatly extended. To be sure, analytical methods are still far from all that can be desired, as is emphasized by the difficulties to be encountered in making carbohydrate determinations, and especially by the misleading results likely to be gained from determinations of types of nitrogen within the plant from other than fresh tissues. But these difficulties are being overcome, and the effectiveness of the whole body of studies on soil nutrients can be greatly forwarded by getting at them from within as well as from outside the plant.

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## MOSAIC AND RELATED DISEASES <sup>1</sup>

L. O. KUNKEL

When Beijerinck<sup>2</sup> learned that he could produce mosaic disease in healthy tobacco plants with juice which had passed through a filter with pores so minute that it held back the smallest bacteria, he believed it was necessary to assume that this strange plant disease is produced by a new kind of causal agent. He therefore formulated his well known hypothesis of a "contagium vivum fluidum." Since Beijerinck's publication of this hypothesis, the mosaic disease of tobacco and many similar diseases of other plants together with the insects that spread them have been distributed throughout the world by commerce in seeds, living plants, etc. At the present time they are of such general occurrence that no one who may wish to study them need look very far in order to find abundant material on which to work. As they have spread from place to place their economic importance has constantly increased. In the United States they have caused and are still causing heavy losses in many important crops. The peach industry, the potato industry, the tomato industry, the sugar industry, the berry industry, and many other agricultural industries of equal importance suffer severe annual losses from virus diseases.

It is little wonder, therefore, that these plant maladies have attracted much attention and that a large quantity of data relative to symptoms and behavior has been accumulated. From a vast amount of literature we learn that as a class they are infectious systemic diseases that develop in immature tissues. They are usually transmitted by cuttings and frequently by seeds. They attack rapidly growing more readily than slowly growing plants, and in every case produce chlorosis or necrosis or both. They are transmitted to healthy plants by juices from diseased plants through wounds, by grafting, and by insects. They can frequently be held in check by growing resistant or immune varieties of plants, by "rogueing" diseased plants out of relatively healthy fields, by keeping fields free from wild host plants, and by controlling insect carriers.

In spite of the extensive studies that have been made and of the valuable knowledge that has been gained, the etiology of the virus diseases still remains obscure. In the light of present knowledge let us consider the

<sup>1</sup> Invitation paper read at the joint meeting of Section G, American Association for the Advancement of Science, the Botanical Society of America, the American Phytopathological Society, and the American Society of Plant Physiologists at Washington, December 30, 1924.

<sup>2</sup> Beijerinck, M. W. Über ein Contagium vivum fluidum als Ursache der Fleckenkrankheit der Tabaksblätter. *Centralbl. Bakt. Par. Infektionskr.* 5: 27-33. 1899.

following questions: Does it still seem necessary to assume a special kind of causal agent? Do the data at hand furnish the basis for a better hypothesis than that formulated by Beijerinck? What lines of study seem most promising at the present time?

Microscopic examination of affected tissues of a large number of mosaic and related diseases of plants has failed to show the presence of bacteria, fungi, or protozoa in sufficient numbers and so distributed as to account for the diseased condition. As a class, they possess a characteristic symptomology which differs from that of diseases caused by known parasites. That many of them are filterable is now well established. There are, therefore, excellent reasons for believing that this kind of plant disease is caused by a special kind of pathogen. Of the several interesting hypotheses that have been postulated from time to time, a few deserve special consideration in this connection.

Because he was unable to transfer certain mosaics including that which attacks species of *Abutilon* except by budding or grafting, Baur<sup>3</sup> concluded that the causative agent must be an inanimate one. He argued that if it were a living parasite it should be transmissible through wounds and by other mechanical means. In the light of what we now know of similar diseases, this argument does not carry much weight. Although some cases have been difficult, most of the virus diseases of plants that have been studied have now been transmitted mechanically or by means of insects. Following Baur's reasoning, we should have to conclude that, while most of them may be caused by a living entity, *Abutilon* mosaic, peach yellows, and a few other similar diseases known to be transmitted only by budding or grafting belong in a special class. It now seems probable that failure to transmit these diseases mechanically is due to lack of knowledge rather than to any special quality of the virus.

A somewhat similar hypothesis advanced by Woods,<sup>4</sup> Freiberg,<sup>5</sup> and others assumes that mosaic diseases may be due to enzymic disturbances. Since, however, there is excellent evidence that the causative agent multiplies within the plant, this conception has not been a popular one.

Perhaps the most widely held hypothesis at the present time is that virus diseases may be due to ultramicroscopic organisms related either to the bacteria or to the protozoa. Opposed to this conception is the fact that all known living organisms come within microscopic vision. We measure them in microns and millimicrons. Since these units are more or less difficult to conceive, it may be worth while to compare them with more familiar ones. In order to do this, let us suppose that all worldly dimensions were increased

<sup>3</sup> Baur, E. Zur Ätiologie der infektiösen Panachierung. Ber. Deutsch. Bot. Ges. 22: 453-460. 1904.

<sup>4</sup> Woods, A. F. The mosaic disease of tobacco. U. S. Dept. Agr. Bur. Plant Ind. Bull. 18: 7-24. 1902.

<sup>5</sup> Freiberg, G. W. Studies on the mosaic diseases of plants. Ann. Mo. Bot. Gard. 4: 175-232. 1917.



to such an extent that an inch would become ten miles. The millimicron would then have a length of about one fortieth of an inch. Objects that are now just visible with the best microscopes would be about four inches in diameter. A very small bacterium such as the influenza bacillus would be about one foot long and about five inches broad. A well developed individual of *Bacillus subtilis* would be sixteen feet long and about one and one half feet broad. In this magnified world bacteria would doubtless be kept in aquaria. If we could visit such an aquarium and could there observe all known species of bacteria, we should find that not one of them would be able to pass freely through a three-inch net. Many thousands of bacteria have been isolated and studied. The isolations have been made without reference to size. Nevertheless, not one has been found that is beyond the resolving power of our best microscopes. The same is also true of the protozoa.

The ultra-microscope makes visible objects that are far beyond the resolving power of the ordinary microscope, but it has not revealed a single ultramicroscopic organism. There seems to be a definite limit to the minuteness of living cells. Our evidence is in agreement with Molisch's<sup>6</sup> conclusion that the existence of ultramicroscopic organisms is highly improbable. Since, however, negative evidence is never fully conclusive, we must continue to search for smaller living cells.

From this brief consideration of hypotheses, it is seen that we do not yet possess a better one than that which assumes a living fluid contagium. When Beijerinck formulated his conception he believed that the virus of tobacco mosaic could pass to some depth into solid agar plates. We now know that he was in error in this respect. The virus does not pass through agar. We are, therefore, led to believe that the causative agent although filterable is nevertheless corpuscular. Beyond the fact that they are "filter-passers" we know little about these corpuscles. They are doubtless very minute but perhaps not ultramicroscopic. Plasticity rather than size may account for their filterability. It would be folly to speculate as to what these corpuscles are like, but I wish to suggest that they are probably of the nature of living cells. With this modification of Beijerinck's hypothesis in mind, let us now pass to a consideration of a few of the many lines of study that seem to offer promise of advancing our knowledge of the virus diseases.

Although of both practical and theoretical importance, comparatively little is known of the host range of even the most common and important virus diseases. But until host relationships are thoroughly understood, we shall hardly be in position to practice intelligent control measures. Hundreds of wild plants show the symptoms of mosaic. How many of these mosaic diseases are related to diseases of important crop plants we do not know.

In cases in which the same virus disease attacks a number of different host plants, we frequently find variations in the symptoms shown on different

<sup>6</sup> Molisch, H. Über Ultramikroorganismen. Bot. Zeit. 66: 131-139. 1908.

hosts. An illustration of this is given by the grass mosaic on the three host plants: Sudan grass, corn, and sugar cane. The mosaic pattern on the leaves and the shape and size of necrotic areas in the stalks differ with each of these hosts. For a full knowledge of symptoms we must know each disease as it affects its different host plants.

It is an interesting question whether virus diseases in their host relationships behave like diseases known to be caused by parasites. The scanty data at present available indicate that as a general rule they attack closely related plants. This behavior is similar to that of such well known parasites as the rusts, smuts, and mildews. There are, however, many striking exceptions to the rule, and grass mosaic may again be used as an illustration. It attacks a wide range of host plants, including many different genera. One of its most susceptible hosts is Sudan grass. The very closely related Johnson grass is, however, apparently immune. Our further studies on host relationships will doubtless yield much of interest and importance.

Another somewhat neglected but very important phase of the virus-disease problem is that which has to do with insect carriers. From the crop-production standpoint the spread of virus diseases is almost entirely an insect problem. It is very important to know the insect or insects that spread each of the different diseases. But we should also know their habits, their insect enemies, their preferences for different host plants, and the conditions under which they become abundant. A more complete knowledge of insect carriers is likely to suggest many new methods of control.

The fact that some virus diseases are transmitted by only one of the several insects that feed on a diseased host plant indicates that the causal agent may pass through certain stages of development in the insect carrier. How long the virus can be carried, whether it has an incubation period in the host insect, and whether the insect itself becomes diseased are important questions. Only a beginning has been made in work on the problem of insects in relation to virus diseases. It is a promising field for further study.

Another line of work offering interesting possibilities is that opened by cytological and histological studies. It has been shown that plastic intracellular bodies are associated with chlorosis in a number of different mosaic diseases. Proof has not yet been brought that these bodies are of etiological significance, or in fact that they represent a living organism. Some students believe them to be waste products resulting from the diseased condition of the plant cell. Such cells undergo many changes. They often contain oil droplets, abnormal plastids, distorted nuclei, crystals, and precipitates of various kinds. It should be understood, however, that the plastic amoeboid bodies associated with mosaic disease bear no resemblance to any of these. They have a structure like that of protoplasm, and they stain like protoplasm. Many of them possess pseudopodium-like appendages. They are vacuolate and at times contain deeply staining granules. They are the most

interesting structures that the method of direct observation has yet revealed. If they are not living organisms it is to be hoped that their true nature will soon be demonstrated by chemical or other means. Their further study offers one of the most enticing phases of the virus-disease problem.

Many other attractive fields for work might be mentioned. Studies on the properties of the virus, chemical studies on diseased tissues, and attempts to isolate and grow the causal agent in pure culture are important subjects for consideration. Enough has been said to indicate the need for work along several different lines. The same methods which have been effective in the solution of other biological problems will no doubt serve to advance our knowledge of these obscure and destructive diseases.

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# THE BIOLOGY AND PATHOLOGY OF SOME OF THE HARDWOOD HEART-ROTTING FUNGI<sup>1</sup>

## PART I

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### INTRODUCTION

The advances made in that phase of forest pathology dealing with wood decay and the heartrots of living trees have been marked by four distinct types of investigation. The first of these consisted of the observations made by the early naturalists, of an entirely superficial character, which may be said to have been brought to a close with the appearance of Theodore Hartig's (10)<sup>2</sup> account of the "red rots" of conifers and hardwoods. The second included the studies of Robert Hartig and the publication of his results in works which clearly recognized the fact that fungi are autonomous organisms, and that they are the cause of the decay observed. It was during this period that the processes accompanying decay were carefully examined and the life histories of the fungi concerned were followed as far as possible. This may be considered the descriptive stage, and its methods are still largely in vogue. The third type of investigation, which is recent and is still in its infancy, is found in Meinecke's bulletin (21). This work brings the studies of the pathologist into focus with those of the forester, both aiming at a practicable regulation commensurate with the highest yield of a controlled forest. The fourth type of inquiry is concerned primarily with the detection of incipient rot in wood which macroscopically appears to be sound. The results of investigations of this type are also applicable to frequent problems in wood-utilization.

With reference to the last category, it will be sufficient for my purpose to emphasize a few of the recent papers. Boyce (3) has described the woods most valuable for aeroplane construction and has pointed out the chief defects likely to occur in these timbers. He emphasized the need of detecting incipient decay, since all such infected wood may be reduced in strength. He comes to the conclusion, however, that "for most purposes it is sufficient to recognize incipient decay as distinguished from other discolorations or defects, without determining the causal fungus."

Kauffman and Kerber (15), and more recently the present writer (1), have

<sup>1</sup> Papers from the Department of Botany of the University of Michigan, no. 218.

<sup>2</sup> References are to literature cited in the bibliography appended to the second paper of this series, to appear in the following issue of the JOURNAL.

dealt with apparently sound wood in certain hardwoods, and have shown that mycelium is present beyond the usual black zone bordering the rotten core and that such wood can not be considered sound.

European investigators have directed their attention to similar problems. Knox and Kaye (16) endeavored to ascertain what measure of practical assistance X-rays would afford to the timber-inspecting staff in their efforts to determine the quality of wood. They hoped that, by an X-ray diagnosis, spiral grain, shakes, incipient decay, etc., would be revealed in aeronautical timber by differences in the density of the wood. Their conclusions may best be given in their own statement:

It can not be claimed for the X-rays when used in examining timber as material that they will tell an experienced and observant timber expert any more than he can deduce from an "outside" visual inspection of either the rough timber or the semi-finished part.

Williamson (37) studied the action of wood on photographic plates. Blocks in a fairly advanced stage of decay were contrasted with sound specimens. No differences were found in the images obtained, except where the disease had progressed so far that the wood was almost powdery.

Thus the question whether wood in the standing tree or in the form of lumber is sound where it appears sound has hardly been touched. The results of a comparative study of a number of wood-destroying fungi and their hosts or substrata in regard to this question are recorded in this paper. In addition, studies concerned with other biological relations between the mycelia of a number of these facultative parasites and their hosts have been made both in the field and in the laboratory.

Deep obligations are expressed for the stimulating suggestions and criticisms of Prof. C. H. Kauffman, under whom I have been privileged to study forest fungi, both in the field and in the laboratory. I desire likewise to express my appreciation to Prof. Filibert Roth for his authoritative advice and his interest in this problem.

## METHODS

### Plants Studied

The wood-inhabiting fungi and their hosts together with the localities from which the trees were obtained are listed here as follows:

*Polyporus hispidus* Fr. on *Fraxinus nigra* Marsh. Michigan.

*Fomes fraxinophilus* Pk. on *Fraxinus americana* L. Michigan, Illinois.

*Fomes fraxinophilus* forma *Ellisianus* forma nov. on *Shepherdia argentea* Nutt. North Dakota, South Dakota, Wyoming.

*Fomes applanatus* Fr. on *Morus rubra* L. Illinois.

*Fomes connatus* Gillet on *Nyssa sylvatica* Marsh., *Acer rubrum* L. Michigan.

*Fomes Everhartii* (Ellis) Gallister on *Quercus velutina* Lam., *Quercus rubra* L., *Quercus alba* L. Michigan, Illinois.

*Fomes igniarius* Gillet on *Fagus grandifolia* Ehrh. Michigan.

*Fomes pomaceus* forma *Crataegi* forma nov. on *Prunus americana* Marsh., *Crataegus* sp. Michigan.

Further studies, in less detail, were also made of some other species, notably the following:

*Fomes conchatus* Gillet on *Fraxinus nigra* Marsh., and on *Crataegus* sp., Michigan; and *Hydnum septentrionale* Fr. on *Acer rubrum* L. Michigan.

### Field Work

The solution of practically all problems in forest pathology depends largely upon the amount of information on hand from the field. Whether the living tree or the product obtained from the forest is the subject of investigation, field work supplies two desirable objects: it opens suggestions as to the methods of attack, and it enables one to make better interpretations of the laboratory tests. Field surveys, which included both infection studies in connection with the heartrots and inoculation experiments with sound trees, have been continuously necessary in this work as a check on the proper correlation of results.

The time devoted to this phase of the work covered a period of several years not only in Michigan, but also in parts of Illinois, Wisconsin, North and South Dakota, Nebraska, Colorado, Wyoming, and Montana. Unless otherwise stated, the trees studied in connection with these investigations were carefully selected and identified in the field by the writer. Notes were taken on the specific silvicultural characteristics of both sound and infected trees. Special attention was given to the occurrence and prevalence of the fungus in any region with particular reference to host relationships.

Late summer and fall were found most convenient for felling the trees. This is because many fungi of this group form sporophores only in those seasons. The fruiting bodies of *Polyporus hispidus*, for example, are found only during a brief period in the fall. The rot produced in the trees was identified by the presence of the fungous sporophores. No rots were determined solely by the appearance of the decayed wood. If it was impracticable to fell the trees at the time when the fungous fruiting bodies were located, the trunks were blazed in order to avoid possible error as to their identity later.

The number of sections cut from the tree after felling depended largely upon the host species and the extent of the rot. The progress of the decay in the wood was traced in the field as far as possible by a macroscopic examination, and the trunk and limb sections were selected accordingly. The wood intended for microscopic study was then brought into the laboratory and was examined while in a fresh condition. Other trees were often sectioned and marked in order to determine the further development of the fungus in the felled trunks. Observations were made on these at appropriate times during the year. This was also done at first to check wood cultures of the fungus growing in the laboratory.

About fifty trees were used for inoculation studies. Various species of

hardwood trees were chosen in order to obtain further information concerning the action of *Polyporus hispidus* and *Fomes pomaceus* forma *Crataegi* on such wood in the field. The inoculation studies, too, were in part used as a check against laboratory tests.

The inoculations were made by transferring the actively growing mycelium from petri-dish cultures to a boring in the tree made with the Pressler increment borer.<sup>3</sup> After the inoculum had been inserted in the bore hole, the wound was covered with grafting wax in order to prevent drying out.

The operations involved in inoculating black-ash sprouts were varied somewhat from those just described for older trees. In this case, longitudinal tangential incisions were first made downward through the bark into the wood so as to obtain a flap of bark still attached at the base. The wood was then inoculated by transferring actively growing mycelium from petri-dish cultures into the wound under the flap. The flap was then firmly bound against the sprout at the region of inoculation.

The procedure used in locating the trees and sprouts both before and after inoculation was an important phase of this work. A rough map<sup>4</sup> was drawn giving the approximate location of the inoculated trees with reference to some more or less permanent object. This was necessary because the wound made by the instrument is not alone sufficient to enable one to find the tree again. Such wounds usually heal rapidly and are soon covered over.

Increment borings were made 14 months later in 5 of the inoculated white-ash trees. The borings were made about three fourths of an inch below the point of inoculation on the trunks. The wood cores obtained from these borings were brought into the laboratory and examined microscopically for mycelium. In the case of the black-ash sprouts, a microscopic examination of the wood was made 4 months after the time of the inoculations.

### Microscopic Observations

The laboratory procedure can be described under three headings: microscopic examination of the materials studied, chemical tests, and culture methods.

In the search for mycelium, wood in all stages of decay was sectioned, both free-hand and with the microtome. The material to be sectioned by an ordinary razor was frequently softened by placing the wood in equal parts of alcohol, glycerin, and water. In such cases, the material was not allowed to stand long enough for the wood to be contaminated by *Penicillium*, *Aspergillus*, or other fungi. In order to understand the character and extent of the mycelium in the wood, transverse, radial, and tangential sections were made. In the cases of some decays, radial sections afforded the best view of

<sup>3</sup> See Graves' "Forest mensuration," pp. 246-248.

<sup>4</sup> It is considered that white paint applied to a small "blaze" is the best way to label individual trees. The paint may be renewed when necessary. Tin tags are likely to be torn off and lost.

the hyphae in the wood. In other cases the mycelium could be traced readily in the vessels and in the other elements in cross sections.

Free-hand sections were found, in general, to answer the requirements as well as or better than those prepared with the microtome. Considering the effects produced on the sections by various reagents necessary in the imbedding processes, and taking into account the time consumed, microtome sections were found to be rather unsatisfactory. For similar reasons, stains such as those recommended for the study of the mycelium by Hubert (12), Williamson (37), and others were used only in special cases. For general purposes, however, eosin was found to be very satisfactory as a reagent for rendering visible the hyphae.

Both decayed and sound wood was examined microscopically soon after the trees had been felled and had been brought into the laboratory. The recently felled tree was found to be in a better condition for sectioning, and the mycelium in the wood was also in a better state for study. In dried-out wood the hyphae of some fungi may become shriveled or affected in some other way so as to be practically invisible. Microscopic observations are thereby made more difficult. This is true, not only from the standpoint of the detection of the hyphae, but also because of the difficulties encountered in identifying the mycelium as distinct from the shredded walls of the wood cells. However, after one has made hundreds of microscopic sections in such a study, the chance of mistaking cell-wall shreds for mycelium disappears with experience. In all cases, however, when doubt existed as to the presence of the mycelium, the specimens were discarded.

### Mechanical Tests

Mechanical tests were employed in the studies of *Polyporus hispidus* on black ash and of *Fomes Everhartii* on red oak. The sound and macroscopically sound wood of these two trees was sawn into blocks and was dried to constant weight in a drying oven. The surface area of the test blocks was obtained by means of calipers. The wood was then tested for resistance to endwise compression on a Riehle machine.<sup>5</sup>

### Chemical Tests

Tests of the amount of lignin and cellulose present in the sound, partly decayed, and rotten wood respectively were made by staining the wood sections with phloroglucin and hydrochloric acid for lignin, and with chloriodid of zinc for cellulose. The use of the Maule treatment<sup>6</sup> and of carbolic acid and hydrochloric acid<sup>7</sup> for lignin tests showed the same general

<sup>5</sup> These tests were made by the writer on the Riehle machine used by the Department of Forestry of the University of Michigan.

<sup>6</sup> A drop of potassium-permanganate solution is placed over the wood sections for a minute. Then hydrochloric acid is added until the color disappears. The wood is then treated with ammonia. Lignified tissues stain red. See Beitr. Wiss. Bot. 4. Stuttgart, 1901.

<sup>7</sup> The sections are exposed to the sunlight for an hour after this treatment. Lignified



results as that of phloroglucin and hydrochloric acid and therefore will not be referred to again.

In order to determine whether the presence of enzymes or the effects produced by them on the wood could be used directly or indirectly to detect the presence of mycelium in the apparently sound wood, methods similar to those described by Zeller (38) were used. Mycelium of *Polyporus hispidus* was grown on wood in liter flasks. After several months, the mycelial mats were removed from the flasks and were dried by an electric fan. The dry mycelium was then ground to a powder in a meat-grinder. Four parts of water by volume with about 1% chloroform were added to this mycelium. After standing for almost 20 hours, the solution was filtered through a Buckner funnel. The enzymes were precipitated from the filtrate by the action of 3 volumes of 95% alcohol. The precipitate was collected on filter paper. Water was then added to this precipitate; the amount added was such that the resulting dispersion was equivalent to 1.5 grams of the powdered mycelium.

Meanwhile a quantity of black-ash sawdust had been prepared from a recently felled tree and soaked for several hours in distilled water. One gram of this sawdust was then placed in each of 3 test tubes. Forty-five cc. of distilled water were added to one tube, and 15 cc. of water and 30 cc. of the enzym dispersion were added to each of the other two tubes. One of the latter tubes containing the enzym preparation was autoclaved. Twenty days later the liquid was filtered from the sawdust, and the sawdust was then boiled in absolute alcohol for about ten minutes, Phloroglycin and hydrochloric acid were added to the alcohol as a test for hadromase. The sawdust was also tested with phloroglucin and hydrochloric acid as well as with chloriodid of zinc.

For comparison, badly decayed wood obtained from a pure culture of *Polyporus hispidus* was placed in absolute alcohol for 15 minutes. This alcohol was then tested with hydrochloric acid and phloroglucin.

Apparently sound red-oak wood was selected from a tree only partially decayed by *Fomes Everhartii*. This wood was treated in a manner like that just described for black ash. The alcoholic extracts were treated with phloroglucin and hydrochloric acid, and the tests were compared.

#### Culture Methods<sup>8</sup>

The cultures were made by the use of portions of very rotten wood, and from the tissue of sporophores. In all cases the cultures were started within a short time after the specimens were brought into the laboratory. The tissues stain green. See West, E. An undescribed timber decay<sup>9</sup> of hemlock. *Mycologia* 11: 226. 1919.

<sup>8</sup> After this paper was ready for the press, my attention was called to the paper by Clara W. Fritz: Cultural criteria for the distinction of wood-destroying fungi. *Trans. Roy. Soc. Canada* III, 17: 191-287. Pls. 1-12. 1923. It is regretted that appropriate comparisons between Miss Fritz's work and my own are not included in this paper.

mycelium from fresh material is most likely to continue growth on artificial media, and the dangers from contamination are considerably lessened.

The sporophores or wood pieces were washed several times in sterile distilled water and were handled with sterile forceps. A sterile scalpel<sup>9</sup> was used to remove the exterior portions from the sections of wood, and the general "tissue method" described by Duggar (5) was employed when sporophores furnished the inoculum. The bits of tissue from the sporophore or from the wood pieces were then carefully inserted on agar plates. The inoculum in some cases was practically a centimeter in diameter. Viable mycelium, as pointed out by Long and Harsch (19), is more likely to be present in a large piece of inoculum than in a small one. In the course of a week or so, growing mycelium appeared on the malt-extract agar. This mycelium was allowed to grow over the plate. These petri-dish cultures were then used as stock material for subcultures.

At first many media such as carrot, oatmeal, and potato agars were used for these initial cultures. Later malt-extract agar was employed almost entirely. Malt-extract agar not only favored a good growth of the mycelium, but it could be prepared readily and in large quantities. After many tests had been made to determine the best concentration of the malt extract, it was found that, within limits, concentration was not as important a factor as might at first seem probable. The amount used influenced chiefly rapidity of growth, but the result obtained depended somewhat on moisture and temperature conditions. For the purpose in hand, however, the following formula was found best adapted for this use:

25 g. agar agar,  
25 g. malt extract,  
1000 cc. distilled water.

In case drying out of the wood pieces was likely to occur before mycelial growth started, the inoculum was first transferred to sterile distilled water before being planted on the agar. This phase of the method was particularly valuable in case cultures were desired from wood macroscopically sound. In such wood there were undoubtedly very few hyphae present, and the addition of water was essential for their continued growth. This process of supplying water to the inoculum resulted in little danger from contamination, and the growth of the fungus was frequently hastened.

Small portions of the growing mycelium were later transferred from the stock petri-dish cultures to wide-mouth Erlenmeyer liter flasks. Each of these flasks contained a slant of the malt-extract agar just described. The slants of agar were prepared by placing 275 cc. of this medium into each liter flask, after which the flasks were sterilized. Before the agar cooled, the

<sup>9</sup> In later work safety-razor blades were found more convenient. These were dipped in corrosive sublimate solution and were heated over a flame until dry just before using. The blades were not heated enough to burn the fungus. This process avoided a delay in waiting for scalpels to cool. Speed was an important factor in such work, since the sporophores were uncovered during the operation.

flasks were placed in a horizontal position in order to allow the medium to form a slant upon solidification. As a result, the layer of agar averaged about  $2\frac{1}{4}$  inches thick at the base. The inoculated flasks were placed in a dimly lighted culture room kept slightly below room temperature. After the mycelium had covered the surface of the agar the cultures were ready to receive the wood blocks, which were then inserted into the flasks.

These blocks were obtained, for the most part, from heartwood, although in some cases sapwood blocks were used. The blocks measured about  $\frac{3}{4} \times \frac{3}{4} \times 2\frac{1}{2}$  inches. The size of the blocks selected was determined by the ease with which they could be transferred under sterile conditions to and from the flasks, and by the necessity of making due allowance for the swelling of the wood when wet. In some species the amount of swelling is greater than in others. Obviously the size of the block also had to do with the possibilities of contamination of the wood; the larger the block, the greater is the danger while handling the material.

After labeling the wood on all faces of the block with a soft lead pencil, the wood was dried in an oven to constant weight. It was found necessary to keep a book record of each block placed in the flasks in order to avoid error. The pencil marks in some species of wood become faint and are not easily seen after the blocks have decayed. Pencil labels on some light colored woods, however, usually show plainly even if the block is considerably affected by rot.

The blocks were placed in large glass capsules, two in a capsule, and about 250 cc. of water was added. The amount of water necessary for each capsule can be arbitrarily determined by sterilization trials with the various species of wood to be tested. The capsules were autoclaved for  $3\frac{1}{2}$  hours at 15 pounds' pressure. The blocks were then removed individually from the capsules and transferred to flasks. For this operation, a long chrome-nickel needle flattened into a spatula form was found most satisfactory. The blocks were easily placed in the flasks by this means and were so disposed on the actively growing mycelium that a maximum amount of space was still available for the further development of the fungus. They could also be placed in any desired position in the flask, *i.e.*, the wood could be inoculated on the transverse or on the longitudinal surface. This arrangement of the blocks was readily manipulated under sterile conditions by the use of the spatulate needle. A small amount of sterile distilled water was then added to each flask.

The flask cultures containing the inoculated wood blocks were opened one year later. The wood, although somewhat firmly attached to the mycelium, was removed without difficulty from its position in the flask by a long wire. The wire was bent in the form of a rake. After the mycelial mats were removed from the wood, the blocks were again dried to a constant weight. The difference between the weight of the decayed blocks and their dried weight at the beginning of the tests was used as one of the criteria for the amount of decay produced.

It will be seen from the above description that the method of procedure during the culture work is somewhat different from methods used by others in the past (13). The blocks are not sterilized in the flasks and later inoculated. Instead, the separately sterilized wood blocks are placed uniformly on pure mycelial cultures of the wood-destroying fungus. The advantages of such modifications involve: (a) The disposition of the blocks in the flasks as desired. (b) The uniform inoculation of the wood blocks. (c) Favorable conditions for an actively growing inoculum. (d) Controllable moisture conditions in the flask.

In the first place, all blocks (unless otherwise stated) were placed in such a position that they were inoculated from the same surface. This is necessary since it has been found that different results may be obtained, depending upon whether the blocks were inoculated on a longitudinal face or on the cross-section ends. Secondly, a uniform inoculation of the wood is obtained by the method employed. In cases in which the blocks have been placed in the flasks, sterilized, and then inoculated, the wood may remain sterile for several weeks before the mycelium reaches the blocks in the culture. Again, some blocks may be badly decayed before the mycelium comes in contact with other blocks. Such difficulties have been noted by Humphrey (13) and by Schmitz and Daniels (28). A third advantage is that the mycelium is well nourished at the time of inoculation. The best conditions for continued growth of the fungus are established by placing the blocks in a particular position. The fourth and very distinct advantage has to do with the moisture relations during the period in which the fungus is grown. Excellent moisture conditions have prevailed, by the use of this method, in year-old cultures without the extra addition of water, so that, when the work was finished, scarcely any loss of volume of the agar had occurred.

Although other agars and media were tried out beside the malt-extract medium described above, the results were not of sufficient importance to be given here. Such media<sup>10</sup> included oatmeal, cornmeal, carrot, potato,

<sup>10</sup> *Oatmeal agar.* This agar was made according to the directions given by Pethybridge and Murphy (Sci. Proc. Dub. Soc. 13: 580. 1913). Ground Quaker oats, 60 g.; cold water, 1000 cc.; agar 3%.

*Cornmeal agar.* This was made by adding 30 g. of cornmeal to 1000 cc. of water; agar 3%.

*Carrot agar.* 400 g. of pared carrots were ground and then soaked in 1000 cc. of distilled water. After standing over night, the liquid was decanted from the carrot pulp. Agar 3%.

*Potato agar.* This was made in a way similar to that described for carrot agar, using about 200 g. of pared potatoes.

*Pectin agar.* The directions outlined by Wolf were followed in making this agar (Studies on the physiology of some plant pathogenic bacteria VII. Pectic fermentation in culture media containing pectin. Phytopath. 13: 382. 1923). A commercial product known as "certo" was used, prepared by the Pectin Sales Co., Rochester, N. Y. An equal volume of 95% alcohol was added to this commercial product. The alcohol caused the precipitation of the pectin in a gelatinous mass. The pectin was collected on cheesecloth in a porcelain filter and was washed with small quantities of fresh alcohol. The pectin was

pectin, Duggar's agar, black-ash sawdust and agar, and various decoctions of sawdust with malt extract.

#### STUDIES WITH *POLYPORUS HISPIDUS*

##### Extent and Description of Visible Decay

In a previous paper (1) the writer gave an account of the decay produced in black ash by *Polyporus hispidus*. It is therefore necessary to present here only a very brief résumé of the more important characteristics of the visible decay. The dark wood of an infected black-ash tree is changed by a partial delignification to a yellowish-brown color much lighter than that of the sound heartwood. The wood is reduced more or less to a soft, spongy mass, which is uniform throughout and which gradually advances into the sound tissues without any sharp distinction or discolorations between the wood first rotted and that in a less advanced state of decay. When a log is split lengthwise, the decayed portion loosens characteristically in cylinder form, especially after the wood has partially dried. As a result, concentric layers may be formed within the shell of that portion of the wood which is still in good condition. Dried cordwood from such trees separates and falls to pieces. This condition may also be seen in white-ash logs attacked by *Fomes fraxinophilus*.

As a rule, no definite black lines separate the so-called rotten and the apparently sound regions of black ash such as are present in trees where the decay is due to *Fomes igniarius* or *Fomes Everhartii*. Bordering lines, however, may occasionally be observed in transverse sections, and irregular dark-brown lines are very noticeable in certain areas of the most rotten wood when sectioned radially. This appearance is due to discolored wood cells that are partially or entirely filled with a dark-brown substance. A mottling effect, consisting of very fine white lines running horizontally, may also accompany the discolorations.

The fungus brings about a delignification of the elements, but the action is not complete. The medullary ray cells in part may indicate the presence of lignin even in the last stages of decay. Like the ray cells, the vessels are also slow in breaking down. The walls or portions of the walls give lignin tests even in the most rotten wood. The wood parenchyma and fibers, when tested for lignin and cellulose, show that a delignification of these elements has taken place. The sections selected for these tests were taken from five trees, including the most rotten areas, the partially decayed wood, and the sound wood. Lignin and cellulose tests made on the partially decayed wood gave results similar to those obtained from badly affected portions of the trees.

then dissolved in warm water and reprecipitated. This process was repeated three times in order to obtain a purer product.  $4\frac{1}{2}$  g. of this pectin were then added to one liter of distilled water containing 20 g. of agar.

### Extent and Effects of the Mycelium

A much greater part of the tree may be infected than is usually assumed or is obvious. In small pockets of even the apparently sound wood, wefts of mycelium often form small mats, usually in the center of the tree. I do not refer to the pith pockets, which persist even in old trees of *Fraxinus nigra*, but to the mycelium which may often be found in such regions bordering the pockets. It is usually necessary to split the logs lengthwise through the center in order that the mycelial mats may be seen, and even then a microscopic examination may be indispensable. Such mycelial mats were found in sections of black ash that otherwise showed no indications of rot and were pronounced sound until they were split open. *Mycelial wefts were found six feet above the area usually designated as rotten.* The wood surrounding the wefts may at first appear sound, but mycelium has been found also in such regions.

In the microscopic study of this apparently sound wood, it was seen that the fungous hyphae do not at first necessarily bring about visible changes in the wood. It was found that, in the initial stages of decay, no discolorations necessarily accompany the presence of mycelium. This fact corresponds to what will be pointed out later in discussing the cultures of this fungus on the wood of black ash, and in the description of the inoculation experiments.

The extent to which this macroscopically sound wood is weakened by *Polyporus hispidus* was ascertained by testing oven-dried blocks for resistance to endwise compression. It must be kept in mind that such mechanical tests are relative, even in sound wood, and are very much more so in partially rotten sections. Wood varies with individual trees of the same species, and in different sections of the same tree. Such variables as the proportion of summer wood (2), the density, the kind and amount of seasoning, etc., must be taken into consideration.

TABLE I. *Compression Tests of Oven-dried Black-ash Wood*

	Block	Size (inches)	Crushing Strength		Crushing Strength per Sq. In.	
			1st Fail	2d Fail	1st Fail	2d Fail
Section with mycelial pocket, but wood apparently sound...	1	0.87 x 1.64	11,780	12,550	8,256	8,795
	2	0.76 x 1.70	10,160	10,610	7,863	8,212
	3	0.84 x 1.69	9,630	9,660	6,783	6,804
Sound.....	1	2.03 x 2.05	39,490	39,620	9,446	9,477
	2	2.00 x 1.62	28,450	29,460	8,780	9,092
	3	1.49 x 1.72	20,000	21,910	7,803	8,198
Most rotten.....	1	0.95 x 1.56	1,910	2,080	1,221	1,403
	2	1.26 x 1.46	1,790	1,830	973	994
	3	1.15 x 1.50	3,190	3,270	1,687	1,730

It will be seen from table I that, when the tests made on genuinely sound wood are compared with those of blocks taken 6 feet from the noticeably

decayed areas in which mycelial pockets were present, the differences in the results are large enough to be taken into consideration; the same is true when the tests from the most rotten sections are compared with those from sound wood. It may be seen that, in general, there is over a thousand pounds' difference per square inch between the first fail for the average of the sound blocks and that of the blocks containing the advanced mycelium.

Beside the facts shown by the detailed microscopic examination of the log sections and the mechanical tests, further evidence to show that mycelium advances beyond the visible rot was obtained from the following experiments: six-inch pieces of freshly cut black-ash sprouts with the bark present, and possessing only sapwood, were washed several times in sterile distilled water. These were dipped in alcohol and were then passed through a flame. Flaming burned off the alcohol and helped to free the surface from organisms. The sprout sections, each lot from one sprout, were then placed in large glass tubes containing sterile agar slants. *Polyporus hispidus* mycelium was then placed on the upper ends of the sticks. After 4 months the wood was removed from the tubes and examined microscopically. Contaminations occurred in some cases, but such cultures were discarded.

Microscopic sections of the interior of each of these pieces of wood were made, starting at the inoculation end and continuing downward until mycelium was no longer found. Mycelium occurred in the vessels of the spring wood; it was apparently well nourished and the hyphae were about two microns in width. Hyphae were also noted in the wood fibers of some sections. The hyphal threads, as seen in this series of sections, had penetrated to a depth of 4 mm. lengthwise of the stick. At this distance the mycelium was difficult to detect because of its extremely hyaline nature and because its width at the apices was not more than 0.5 micron. This is the type of mycelium that is difficult to detect in apparently sound wood. No discolorations accompanied the presence of the hyphae in these "advanced" areas in the sticks. Mycelium was also found in the pith cells. The occurrence of hyphae at this place was to be expected, since the cells examined were near the point of inoculation.

Further experiments were conducted in an attempt to demonstrate the effects of mycelium upon wood in the initial stages of decay. These were based upon the methods described by Czapek (4) in the study of wood destroyed by *Merulius lacrymans*. Czapek extracted hadromase directly from the decayed wood by the use of alcohol. He found that the alcoholic extract from the rotten wood gave a red color with phloroglucin and hydrochloric acid. Only a relatively small amount of hadromase was obtained by him from sound wood.

By applying Czapek's method to black-ash wood, could one distinguish the apparently sound wood from the genuinely sound wood? Tests were made with sound and badly decayed wood, and all the results compared. Although the tests were repeated several times, the results were not con-

clusive. The alcoholic extracts obtained from both the sound and the apparently sound wood gave a red color when treated with phloroglucin and hydrochloric acid. Judging by the depth of color obtained, it may be said that not even a degree test was shown by these experiments. Similar negative results were obtained with alcoholic extracts from solutions containing the enzym liquids as well as from the controls. Therefore, and unfortunately, such tests can not be relied upon to detect the initial stages of decay in black ash in the case of its heartrot, and perhaps this is also true for other heartrots.

### ***Polyporus hispidus* in Culture**

*Polyporus hispidus* was successfully grown on various kinds of wood (Pl. LVIII) as well as on a great variety of other substrata. The growth of the mycelium on wood will be described first. The selection of the wood species used in this culture work was largely based upon the facts known concerning the growth habits of the fungus in nature. Black ash was chosen because this tree is the principal host species for *Polyporus hispidus* in Michigan. White ash and red oak were used in order to study the possibilities of infection and the type of decay of these woods. The writer (1) has pointed out that no fruiting bodies of this fungus have been reported on *Fraxinus americana* or on *Quercus rubra* in Michigan. In addition to the ring-porous types such as ash and oak, it seemed desirable to use also a diffuse-porous wood; for the latter type yellow birch was selected. Apple wood was used because the apple is one of the chief hosts for this fungus in Europe.

Including controls, 44 large Erlenmeyer flask cultures were used in this study. The large number of flasks was prepared in order to have additional cultures to substitute should contaminations occur; however, very few cultures were contaminated. The results reported here were all obtained from cultures free from other organisms. These cultures were examined and studied at various intervals throughout the year. It was found, however, that the characteristics of the fungus in culture did not appreciably vary throughout the period in which they were kept growing. The results obtained from these cultures were recorded after the fungus had been kept growing for 12 months.

No special difficulty was experienced in inoculating the wood blocks, and all the kinds of wood used became infected a short time after they were placed in the flasks. The color of the mycelium on the different woods was practically the same in all cases, namely "antimony yellow" to "buckthorn brown" (Ridgway). It was found, however, that the manner in which the fungus acted on the various wood blocks was more or less specific for each particular kind of wood. That is to say, the character of the external mycelial growth of *Polyporus hispidus* was fairly constant for all species of wood, but its effects on the wood elements were somewhat different. This growth behavior agrees, as will be seen later, with the studies made on the blocks after they were removed from the flasks.



The mycelium in the white-ash cultures produced a somewhat flocculent growth which was luxuriant enough to fill or partly to fill the flasks. "Filling the flask" refers chiefly to a thin mycelial layer growing upon the interior surface of the glass; the amount of mycelium, of course, depended to some extent on moisture conditions. The amount of moisture, however, was the same in all the flasks as far as it was possible to regulate this factor. The mycelium growing on the black-ash and yellow-birch blocks had a quite compact and tough appearance. On yellow-birch cultures, however, the mycelium in a few cases partly filled the flask in the manner described for white ash. On the apple wood it produced a growth more nearly resembling that on the white-ash cultures. The mycelium covered the oak wood sparsely as compared to the completely enveloped white- and black-ash, yellow-birch, and apple blocks. In the majority of the oak cultures, the blocks were covered only by a thin arachnoid layer.

Macroscopically the wood blocks showed decided evidences of decay after the mycelial mats had been removed. In general, the manner and extent of decay varied greatly with the species of wood, but was more or less nearly constant for any one kind of wood. This is of special interest since very few comparisons of the types of hardwood decay, produced under such controlled conditions, have been recorded. Zeller (39) studied resin-impregnated yellow-poplar blocks in which decay was produced by *Lenzites saepiaria* and by *Polystictus hirsutus*. He was concerned only, however, with the relation of resin to the decay produced by these two fungi.

The decay produced in the white-ash blocks, after one year in culture, is first of all characterized by a chalky appearance of the wood. The surface of the blocks rubs off between the fingers, and the wood may be readily crushed by pressing. Dark-brownish lines on all six faces of the blocks are prominent macroscopic features of the decomposition of this wood. Because of the biological significance of these lines in regard to wood decay, their formation on culture blocks will be discussed in greater detail later. It is difficult to determine with a hand lens whether the spring or the summer wood was most resistant to decay in some blocks. The majority of blocks, however, showed that the spring wood was attacked most severely.

The ray cells, as seen in microscopic sections, remained more strongly lignified than the other elements, although the delignifying action was not complete in any one case. Radial, tangential, and cross sections of the wood exhibit much the same reactions as black-ash wood decayed by *Polyporus hispidus* in nature.

Since wood is one of the most widely used materials for construction, information concerning its decay resistance is of special importance. No extensive work has dealt with resistance to decay of hardwoods under controlled conditions. With this fact in mind, the following tests on white-ash wood were made. Decay-resistance tests made on the other hardwoods mentioned will be presented as the various species are discussed. Loss in

oven-dry weight of the blocks is used to designate the amount of decay produced by the fungus in one year. All the blocks were weighed on a fine analytical balance to the third decimal place.

TABLE 2. *Decay Resistance of White-ash Wood Inoculated with Polyporus hispidus*

No. of Block	Oven-dry Weight in Grams before Test	Oven-dry Weight in Grams after 1 Year	Loss	Loss %
1.....	12.995	10.882	2.113	16
2.....	12.010	9.993	2.017	16
3.....	10.728	7.798	2.930	27
4.....	13.182	11.037	2.145	16
5.....	11.388	9.958	1.430	12
6.....	12.351	8.275	4.076	33
7.....	14.552	12.447	2.105	14
8.....	13.326	8.812	4.514	34
9.....	11.393	9.746	1.647	14
10.....	10.093	8.670	1.423	14
11.....	12.169	10.030	2.139	17
12.....	9.684	7.360	2.324	24
13.....	10.346	8.550	1.796	17
14.....	10.139	7.560	2.579	25
15.....	9.467	7.975	1.492	15
16.....	10.466	7.450	3.016	29
17.....	10.197	8.635	1.562	15
18.....	13.615	10.668	2.947	21
19.....	12.512	10.860	1.652	13
20.....	12.535	10.829	1.706	13
21.....	13.090	11.240	1.850	14
22.....	14.278	11.877	2.401	17
23.....	12.251	8.999	3.252	26

Average loss %.....19.2

Average loss % for 5 control blocks..... 2.6

Table 2 shows that the average loss in weight of white-ash wood due to decay produced by *Polyporus hispidus* is 19.2%. It is to be noted that 8 of the heartwood blocks selected from the same tree showed an amount of decay greater than that indicated by this average. The greatest difference in the decay resistance of this wood is noted in the cases of blocks 6 and 8. Such variations might be readily accounted for if they occurred in cultures where the inoculum was placed in the center of the flasks after the flasks and wood were sterilized together. But in the cultures referred to in table 2, all the blocks were uniformly inoculated. There is also a difference of 22% in the rate of decay as between some of the individual blocks. To be sure, the amounts of spring and summer wood must in part account for some of the differences, but the blocks were selected so that the proportions of summer and spring wood were as nearly similar as possible. The losses expressed in percentage for the various blocks clearly show that results based upon individual cases do not indicate the average decay resistance of the wood species tested.

The action of the fungus on black-ash wood may be compared with that on white ash, since the decay produced in both species was very similar. In the white ash, the decay produced by this fungus was slightly more (2.1

percent) than in its common host in Michigan, *Fraxinus nigra*. Dark-line formation was less pronounced in black ash, and the wood was less light in color. The color of the normal wood of black ash, however, is much darker than that of normal white-ash wood. In most respects, the decayed blocks had the general appearance of sections of rotten wood taken from standing trees. The spring wood in the blocks was somewhat more reduced than the summer wood, giving the latter the appearance of being the more resistant. Upon a microscopic examination, mycelium was found abundantly distributed in all the cells of the black-ash blocks, and lignin and cellulose tests demonstrated the general delignifying action of the fungus. The medullary rays and vessels were apparently the last to break down, as parts of these cells indicated the presence of lignin even in the most rotten blocks.

Decay-resistance tests similar to those with white ash were made on 25 blocks of the wood of *Fraxinus nigra*. The results are given in table 3.

TABLE 3. *Decay Resistance of Black-ash Wood Inoculated with Polyporus hispidus*

No. of Block	Oven-dry Weight in Grams before Test	Oven-dry Weight in Grams after 1 Year	Loss	Loss %
1.....	6.938	5.837	1.101	16
2.....	7.106	5.364	1.742	24
3.....	6.000	4.789	1.211	20
4.....	4.978	3.965	1.013	20
5.....	7.191	5.950	1.241	17
6.....	6.023	4.702	1.321	22
7.....	11.601	8.519	3.082	26
8.....	10.433	8.770	1.663	16
9.....	13.472	11.657	1.815	13
10.....	12.546	8.789	3.757	30
11.....	13.855	12.045	1.810	13
12.....	9.489	7.346	2.143	22
13.....	13.562	10.434	3.128	23
14.....	13.368	9.580	3.788	28
15.....	10.120	8.995	1.125	11
16.....	12.037	10.828	1.155	9
17.....	12.672	11.454	1.218	9
18.....	13.685	12.211	1.474	11
19.....	11.960	9.942	2.018	17
20.....	13.190	11.138	2.052	15
21.....	7.279	6.535	0.744	10
22.....	11.316	9.224	2.093	18
23.....	10.301	9.434	0.867	8
24.....	10.570	8.791	1.779	17
25.....	8.720	7.570	1.150	13

Average loss %.....17.1

Average loss % for 3 control blocks..... 0.7

The results obtained from this series of tests show that the average loss in weight is not very different from that in the case of white ash.

All the yellow-birch blocks showed decided evidences of decay. The light color of the sound yellow-birch wood was converted to a yellowish-brown color by the fungus. The difference between sound and decayed

blocks was even more noticeable here than in ash. In normal yellow-birch heartwood the definition of the annual rings is usually not very sharp because of its diffuse-porous structure. In the decayed blocks, however, the rings were plainly marked. The pores of the summer wood formed decided ridges in contrast to the more reduced spring wood. The occurrence of dark lines in all cultures was especially characteristic of the decay produced in yellow birch. Lignin and cellulose tests were made on both sound and decayed wood. It was found that, in general, the action of the fungus was that of a delignifier. Slight lignin reactions, however, were obtained in the most rotten blocks, the vessels and medullary ray cells giving the clearest reactions.

Decay-resistance tests were likewise made in the same way on the yellow birch. The results are recorded in table 4.

TABLE 4. *Decay Resistance of Yellow-birch Wood Inoculated with Polyporus hispidus*

No. of Block	Oven-dry Weight in Grams before Test	Oven-dry Weight in Grams after 1 Year	Loss	Loss %
1.....	13.792	11.154	2.638	19
2.....	14.562	7.902	6.660	45
3.....	16.687	12.339	4.348	26
4.....	14.480	8.244	6.236	43
5.....	15.601	12.741	2.860	18
6.....	15.308	9.268	6.040	39
7.....	12.374	9.264	3.110	25
8.....	13.554	11.330	2.224	16
9.....	16.838	12.702	4.136	24
10.....	15.365	11.895	3.479	22
11.....	16.764	13.967	2.797	16
12.....	16.295	12.440	3.855	23
13.....	13.095	8.412	4.683	36
14.....	17.742	14.074	3.668	20
15.....	16.923	13.946	2.977	23
16.....	16.546	10.086	6.460	39
17.....	16.590	12.382	4.208	25
18.....	15.466	10.060	5.405	34
19.....	15.958	13.841	2.117	13
20.....	13.489	10.951	2.538	19
21.....	14.120	11.951	2.169	15
22.....	13.635	9.675	3.960	29
23.....	13.022	11.192	1.830	14
24.....	16.509	14.445	2.064	12
25.....	15.419	12.769	2.650	17

Average loss %.....24.4

Average loss % for 4 control blocks..... 0.0

While yellow birch is one of the best timber trees, it is not considered a durable wood. The figures of table 4 confirm this fact as it applies in nature. It will be noted that the loss percent of yellow birch averages 24.4. This loss is much greater than that obtained for any of the other wood species inoculated with *Polyporus hispidus*. Block 2 shows a loss in weight of 45 percent; block 4, of 43 percent. Not only is the rate of decay greater in yellow birch than in the other woods tested, but there is also a greater

variation between individual blocks. Table 4 shows that there is a difference of 33% in decay between the blocks 2 and 24 in this series.

The oak blocks, as was to be expected, were plainly the most decay-resistant of all the hardwood species tested. The actual condition of the wood, however, could not be judged altogether by the fact that there was a scant mycelial growth on the surfaces of the blocks. It has been found in some cases that a greater penetration of the wood may be accompanied by a small amount of surface mycelium. The blocks weighed little less than sound wood one year after they had been inoculated with *Polyporus hispidus*. The oak wood was not soft when dried, as was the case with the other woods already discussed. The blocks had undergone no color changes, and no black or dark lines had been formed. The formation of these lines apparently depends somewhat upon the wood, and therefore is not, as is usually supposed, necessarily characteristic of a given rot.

TABLE 5. *Decay Resistance of Red-oak Wood Inoculated with Polyporus hispidus*

No. of Block	Oven-dry Weight in Grams before Test	Oven-dry Weight in Grams after 1 Year	Loss	Loss %
1.....	8.649	7.185	1.464	17
2.....	8.708	7.650	1.058	12
3.....	7.137	5.492	1.645	23
4.....	6.916	6.588	0.328	4
5.....	7.325	6.512	0.813	11
6.....	7.173	6.250	0.923	13
7.....	6.807	5.774	1.033	15
8.....	7.958	7.547	0.411	5
9.....	7.955	7.442	0.513	6
10.....	8.643	7.835	0.808	9
11.....	7.096	5.830	1.266	18
12.....	7.321	6.522	0.799	11
13.....	7.491	5.325	2.166	29
14.....	7.070	6.418	0.652	9
15.....	6.806	6.265	0.541	8
16.....	8.785	8.143	0.642	7
17.....	8.910	8.262	0.648	7
18.....	8.412	7.815	0.597	7
19.....	8.646	7.905	0.741	8
20.....	8.690	7.982	0.708	8
21.....	6.927	6.320	0.607	9
22.....	7.864	7.236	0.628	8
23.....	8.589	7.635	0.954	11
24.....	7.987	7.435	0.552	7
25.....	8.592	7.895	0.697	8
26.....	8.639	7.899	0.740	8
27.....	6.855	6.527	0.328	5
28.....	6.768	5.886	0.882	13

Average loss %.....10.5

Average loss % for 5 control blocks.....3.1

The mycelium, where thin mats had formed on the surface, became loosened from the blocks in a peculiar manner. Mycelial strands, attached to the aerial hyphae, had grown into the large vessels of the wood, producing small holes. Their length varied from less than 1 mm. to  $\frac{3}{4}$  cm. These

enlarged holes in the wood and the intact and projecting medullary rays were the chief macroscopic features of the decayed wood. Mycelium was found in all the elements, and its general delignifying effect was shown by lignin and cellulose tests.

The results obtained from the decay-resistance tests made on red oak serve as an index to the amount of decay produced by *Polyporus hispidus* on the wood of this species in one year. The results are recorded in table 5.

A comparison of table 5 with the preceding series of tests shows that the rate of decay was much slower in red oak than in either white or black ash. The loss in weight due to decay was less than half the amount produced by *Polyporus hispidus* in yellow birch. The results agree well with the facts indicated by the macroscopic examination of the cultures, *i.e.*, the small amount of mycelial growth corresponds with a lesser degree of decay. It must not be forgotten that at times oak posts or lumber lying in moist situations decay with exceeding rapidity when acted on by fungi which usually affect oak wood. In this case, however, the data were obtained from an ash fungus and not from an oak-destroying organism.

TABLE 6. *Decay Resistance of the Wood of Cultivated Apple Trees Inoculated with Polyporus hispidus*

No. of Block	Oven-dry Weight in Grams before Test	Oven-dry Weight in Grams after 1 Year	Loss	Loss %
1.....	11.182	9.513	1.669	15
2.....	8.600	6.915	1.685	20
3.....	11.920	9.664	2.256	19
4.....	12.130	9.672	2.458	20
5.....	11.552	9.620	1.932	17
6.....	12.041	9.884	2.157	18
7.....	16.751	14.649	2.102	13
8.....	8.472	7.097	1.375	16
9.....	13.146	11.377	1.769	13
10.....	10.890	9.100	1.790	16
11.....	8.437	7.378	1.059	13
12.....	10.681	9.127	1.554	15
13.....	11.494	9.766	1.728	15
14.....	10.144	8.359	1.785	18
15.....	9.395	7.945	1.450	15
16.....	9.103	7.264	1.839	20
17.....	16.977	13.801	3.176	19
18.....	9.560	8.118	1.442	15
19.....	11.270	9.775	1.495	13
20.....	9.008	7.842	1.166	13
21.....	9.272	8.111	1.161	13
22.....	9.145	7.683	1.462	16
23.....	8.531	6.938	1.593	19
24.....	9.171	7.873	1.298	14
25.....	7.240	6.214	1.026	14

Average loss %.....15.9

Average loss % for 5 control blocks.....0.14

In the apple blocks the mycelium changed the exterior parts of the blocks to a crumbly, whitish-yellow mass of decayed wood. The reddish color in

the interior, although much lighter, more nearly resembled that of the sound tree. Irregular and winding dark-brown lines occurred in the blocks just as Prilleaux (26) has described for the most rotten wood of fruit trees affected by this fungus in Europe. The appearance of the rotten blocks is distinctive in that the fine rays of the apple wood show a lamination, *i.e.*, the rays stand out by themselves because of their greater resistance, while the elements between these cells are partially or completely eaten away. That the medullary rays are the last to be attacked may also be seen by microscopic sections of the rotten wood. In the most disorganized areas, these cells retain their form even though they are very badly corroded. Lignin and cellulose tests of such sections also demonstrate the resistance of the ray cells.

Decay-resistance tests were likewise made on the apple wood. The results are given in table 6.

This table shows that the loss in weight of the apple wood in cultures, due to *Polyporus hispidus*, is 15.9%. This loss more nearly corresponds with the results obtained for black-ash wood than with those for any of the other woods tested. Black ash is only 1.2% more resistant to decay than apple. This correspondence, it may be noted, falls in with the facts that black ash is the only host reported for the fungus in Michigan and that it is certainly a common tree most commonly infected naturally, while the apple tree is known to be a common host for the fungus in Europe. It is notable that the rate of wood decay produced by *Polyporus hispidus in vitro* corresponds with that occurring in nature.

The experiments above described show that this fungus is capable of infecting the heartwood of various kinds of trees. In order to obtain information concerning the ability of the organism to attack sapwood, both heart- and sapwood cultures of the fungus were compared. The wood for this study was selected from a young healthy black-ash tree. Wood sections from this black ash were brought into the laboratory, where small blocks were immediately sawn out of the heartwood and of the sapwood. These blocks were handled according to the methods described on pages 529, 530. Sterile water, however, was placed in the test tubes instead of agar. Pure cultures were obtained which confirm the field observations concerning the ability of the fungus to attack sapwood. The sapwood, to be sure, had been removed from the tree, but the outdoor inoculation experiments described later verify the facts found in the cultures.

#### Production of Fruiting Bodies of *Polyporus hispidus* in Culture

It was not the purpose of the writer to study the conditions necessary to produce fruiting bodies of wood-rotting fungi on artificial media. In several of the cultures, some of these fungi did, however, show initial tube-formation on the thicker masses. One culture of *Polyporus hispidus* developed such a pore surface.

It is interesting to note that this initial and incomplete fruiting body, similar to those that were formed in many of the cultures of the other wood-inhabiting fungi studied, appeared only on the upper portions of the agar slant. Cases like this have been previously reported by other investigators, notably by Long and Harsch (19). In an endeavor to explain this phenomenon with reference to *Polyporus hispidus* in particular, the following experiments were set up:

I. It was decided to determine the effects of drying upon fruiting-body formation, since the agar in many cultures was obviously dried in the upper portions of the slants. To this end, cultures of *Polyporus hispidus* on malt-extract agar were allowed to dry to some extent. No fruiting bodies even of the so-called abortive type appeared in these cultures. The drying of the medium in itself can not account for the formation of fruiting bodies in this fungus.

II. An experiment was undertaken to study the starvation effects upon the fungus produced by a lack of food and moisture. A reduction in the essential food materials has been shown to bring about reproduction in certain plants. For example, Miss Wakefield (34), in her studies on fruiting-body formation in the Hymenomycetes, induced *Schizophyllum commune* and *Stereum purpureum* to form sporophores by reducing the food supply. With her results in mind, a series of cultures were prepared. Inoculations with actively growing *Polyporus hispidus* mycelium were made on agar and then on media containing various concentrations of malt extract. No growth of the fungus occurred on the agar alone. Although the mycelium grew luxuriantly on the majority of the other agars, no fruiting bodies were produced either in a series kept in the dark or in those in the light. Transfers were made from cultures having a large percentage of food material to media containing less malt extract. No fruiting bodies were formed in these subcultures. Cultures of a third type were made in which the mycelium was allowed to dry in the flasks. Then sterile blocks, some previously water-logged and others soaked in a concentrated malt-extract solution, were placed on this dried mycelium. Vegetative growth of the fungus was soon resumed, and all blocks were covered with a luxuriant growth of mycelium. No indications of fruiting-body production occurred. In other cultures, dried mycelium of the fungus was taken from old cultures and placed on sterile wet blocks of black-ash wood in mason jars. Here also the mycelium resumed growth, but there was no tendency toward fruiting-body formation.

III. The effects of light on *Polyporus hispidus* cultures were observed in the following experiment. Large test-tube cultures were placed in both vertical and horizontal positions, one series in the light and a second series in a dark room. Aside from the fact that the mycelium grown in the light was darker brown, no appreciable vegetative differences were noted in the cultures. Other tubes of *P. hispidus* were placed in the light in a vertical



position with black opaque paper around the cultures. Light fell directly on the tops of and parallel to the tubes but could not enter directly from the sides or from the bottoms. Long and Harsch (19) found that small but otherwise typical pilei were produced in their cultures by this method. *Polyporus hispidus*, however, did not form fruiting bodies in such cultures.

IV. Various kinds of media were used for cultures of *Polyporus hispidus*. The fungus grew rapidly on malt-extract agar, oatmeal agar, carrot agar, and Duggar's agar, and it was grown on cornmeal agar, pectin agar, black-ash sawdust, and on various decoctions of sawdust with sugars. With the exception of pectin agar, on which the fungus made very little growth, the uniformity of the vegetative mycelium on all these media is striking. The rate of growth varies somewhat with the kind of substratum, but in general the differences occurring are very slight. No fruiting bodies were formed in any of the substrata mentioned. This observation agrees with the results of studies made by White on *Fomes applanatus*. White (36) found that the latter fungus did not fruit on any agars tried, and that it was necessary to complete the cultures on wood in order to secure fruiting bodies.

Small hemispherical protuberances were formed in many cultures, resembling the initial stages of fruiting-body formation. In one case, the exterior portions of these protuberances took on a hispid-hairy character like that found on the surfaces of fruiting bodies produced in nature. Definite tubes were produced in this case. As growth proceeded, however, these became stuffed with mycelial threads. In microscopic sections, large brown cystidia were seen, measuring  $9 \times 51$  microns. No basidia or basidiospores were found in these tubes.

#### Field Inoculations

Although *Polyporus hispidus* was successfully grown in the laboratory on both heartwood and sapwood, it was thought desirable to determine whether the fungus could be made to grow on living trees by artificial inoculation, especially on an unusual host. Twenty-five white-ash trees were inoculated for this study with laboratory-grown mycelium of *Polyporus hispidus*. After 14 months, these white-ash trees, inoculated with mycelium obtained originally from *Fraxinus nigra*, were examined and studied. At this time, no fruiting bodies of the fungus or evidences of their former presence were observed and there were no outward indications of decay. This was to be expected. The fungus is rather slow in its growth even when grown in culture in the laboratory under favorable temperature conditions and with the optimum supply of food and moisture.

Since there were no external effects produced by the inoculation, attention was directed to the interior conditions of the trees. Increment borings were made about three fourths of an inch below the inoculation wounds on 5 of the white-ash trees. The core pieces were carefully removed from the auger, placed in separate envelopes, and labeled. They were

brought into the laboratory and examined microscopically. Mycelium was found in the cores of all 5 trees inoculated, thus pointing to the fact that *Polyporus hispidus* is able to infect white-ash trees. These experiments show that, under conditions favorable to infection, other hosts than the usual ones may without doubt be occasionally attacked by this fungus.

Although little can be said as to the effect produced on the wood by this "advanced" mycelium, the condition of the cores as such is of special importance. Only an occasional hyphal strand was observed, since the mycelium was not uniformly distributed throughout the wood. Mycelium was found in the vessels in 4 of the 5 cores and in the medullary ray cells in the fifth. This corresponds, in general, to what Mayr (20) found in his work on *Polyporus betulinus* and *P. laevigatus*. He observed that the mycelium spreads in a longitudinal quicker than in a radial direction, and that the vessels are the path of advance. In my work, the greatest distance to which the mycelium had penetrated into the wood from the source of inoculation could not be determined. It may, however, be said that in all the 5 trees the mycelium had progressed at least three fourths of an inch in 14 months. Mayr found in his studies that the mycelium had grown  $2\frac{1}{2}$  cm. from August to November. Münch (22) allowed his infected trees to stand for  $3\frac{1}{2}$  years before felling. During this time the mycelium of *P. igniarius* had progressed 33 cm.

No color changes were observed in the cores taken from the white-ash trees, and the presence of the few hyphae in the cells could be determined only with the microscope. This fact corresponds to what has been found regarding the presence of mycelium in apparently sound wood—macroscopically like normal sound wood, but containing mycelium. It is a significant fact that the mycelium may not produce outward signs of rot during the early stages of infestation. Many instances of the presence of mycelium in wood macroscopically sound have been studied in the course of this work. These will be described in their proper relation.

Additional inoculation experiments were made on black-ash sprouts. It was found that the rot caused by *Polyporus hispidus* in a standing tree is mainly confined to the upper part of the bole. In connection with control measures, one may ask whether it is possible for sprouts to become infected. In order to answer this question, black-ash sprouts 13 to 19 years old were inoculated. No heartwood had been formed in these plants.

Four months later, 5 of the inoculated sprouts were brought into the laboratory for microscopic study. It was found that the mycelium had penetrated the cells of 4 of the sprouts and was in an actively growing condition at the time of examination. These experiments show clearly that black-ash sapwood, under the conditions stated, can be infected by *P. hispidus*. Whether the mycelium will continue its growth in the sapwood at some distance from the wound, or whether the fungus will later succumb in consequence of the cell activities of the young sprouts, can be answered only by a continued study of other inoculated sprouts left for a long enough time in the field.

STUDIES WITH *FOMES EVERHARTII* AND *FOMES IGNIARIUS*

The decay produced in hardwoods by *Fomes Everhartii* is well known, although it has never been fully described. This white woodrot is said to be almost indistinguishable from the decay caused by *F. igniarius* in frondose trees. Earlier studies of this rot, made by Von Schrenk, Spaulding, Hedgcock, and others have been confined largely to host records. Hedgcock reports that this fungus, next to *Polyporus dryophilus*, causes the most important rot of oaks. He records the fungus on fifteen species of oak and states that it causes a heartrot of walnut, mesquite, beech, and birch. Weir lists *Populus trichocarpa* also as a host for *Fomes Everhartii*.

In Michigan this rot, as regards oaks, outranks in importance the rot produced by *Fomes igniarius*. Like *Polyporus hispidus*, this fungus shows interesting host relationships. *Fomes Everhartii* is often epidemic on *Quercus rubra* and *Q. velutina*, occurring in the southern wood-lots as well as in the sand plains farther north in the state. Epidemics of this fungus on black oaks have been found usually in wood-lots bordering streams, where favorable moisture conditions exist. Infected trees of *Quercus alba*, on the other hand, are more difficult to locate. Although white oak is associated in such areas with *Q. velutina*, it is not so commonly attacked. The trunks of a large percentage of black oaks may show large, gnarly burls bearing numerous fruiting bodies of the fungus. White oaks in the same stand may show no such outward indications of decay and may be entirely free from the fungus. However, diseased white-oak trunks do occasionally show similar swellings, containing not only mycelium but also rudimentary fruiting bodies or mature sporophores similar to those on infected black-oak trees.

Surprisingly few cases have been found in which *Fomes Everhartii* continues its growth after the trunks, limbs, or logs are down. Even where the epidemics occur, fruiting bodies are seldom produced on oak wood lying on the ground. I have, however, found this fungus in an actively growing condition on logs of both white and black oak. Even though the fungus may continue growth under conditions favorable to development, the fruiting bodies are most generally found on living oaks. Such field observations point toward a more or less definite biological relation between this fungus and its host. The fact that *F. Everhartii* is capable of attacking sapwood, as will be seen from the description of the rot, corroborates these observations.

## Extent and Description of the Visible Rot in Oaks

Cross sections of trunks affected by this white rot show that the decay is by no means confined to the center of the trunk. Rotten areas often develop on one side of the trunk and apparently advance toward the bark instead of attacking the remaining heartwood. The rot may occur in any part of the trunk or limbs and may even extend into the roots. The fungus attacks both the heart- and the sapwood. The entire bole may be uniformly decayed, or the rot may appear more or less localized. In most oak trunks the

rot, at its upper and lower limits, is in the form of narrow cones whose bases meet at the region of greatest decay. The region occupied by the rot depends upon growing conditions for the tree, the point at which the fungus entered, and the length of time the tree has been infected. However, field observations indicate, in general, that the more extensive areas of visible decay occur commonly in the trunk only a few feet from the ground.

Stem analyses made in different years on red- and on white-oak trunks strengthen the field observations on this point. With only one exception, these trees were obtained from different woodlots near Ann Arbor. It will be seen from table 7 that, in 5 out of 6 trees, the largest rot areas are not far from the bases of the trunks. However, the entire bole may show indications of decay.

TABLE 7. *Extent of Visible Decay in Red and in White Oaks Infected with Fomes Everhartii*

No. of Tree	Name	Trunk Diameter at Base of Tree; also Presence or Absence of Burl	Height above Ground at which Decayed Area has Largest Diameter	Height to which Rot Extends in Standing Tree	Downward Extent of Rot below Area of Greatest Decay
1.....	<i>Quercus alba</i>	9 inches. No burl	5 ft.	12 ft.	To ground.
2.....	<i>Q. rubra</i>	9 inches. No burl	6½ ft.	7⅙ ft.	4 ft. from ground.
3.....	<i>Q. alba</i>	6 inches. Burl present	5 ft.	18 ft.	3 ft. from ground.
4.....	<i>Q. rubra</i>	13 inches. Burl present	2 ft.	9 ft.	Extending into main roots.
5.....	<i>Q. alba</i>	6½ inches. No burl	22 ft.	24½ ft.	9 ft. from ground.
6.....	<i>Q. rubra</i>	9 inches. Burl present	4 ft.	35 ft., extending into branches	To ground.

The spring wood is destroyed first by the fungus, and ultimately the dense bands of summer wood are dissolved. The wood substance is eventually reduced to a soft and crumbly consistency. However, the trunks do not become hollow.

In transverse sections of the most rotten wood, the medullary rays stand out in prominent relief. The cells between the rays may be partially or entirely destroyed by the fungous hyphae. The mycelium attacks all elements of the wood and has a delignifying action upon the walls of the wood elements. If an infected log is split lengthwise, the whitish portions and, to a lesser extent, the areas surrounding the badly decayed parts have a somewhat shredded appearance.

The outline of the decayed area in a cross section of the trunk is usually irregular and especially so in the swollen areas where trunk burls accompany the rot. No concentric black lines limit this irregular area, although the badly decayed regions are frequently bordered by a discolored zone of wood. The wood in this stained area may be macroscopically similar to the sound wood excepting in color. To a certain degree, at least in some cases, these

discolorations disappear after the wood has been in the laboratory for a few years. In other oak trees studied, the wood bordering the most rotten portions was sound to all appearances when the tree was first felled. Later, a light brownish discoloration appeared outside the visibly rotten parts. Lignin and cellulose tests gave indications that mycelium was present and had acted on this wood outside the badly decayed areas. Such tests showed that a dissolution of the lignin had already taken place to a small extent.

### Extent and Effects of Mycelium

Although the wood of all the oaks was examined microscopically for mycelium, tree no. 3 (table 7) was especially well adapted for this study. Seven feet above the ground, the visible decay was confined to the spring wood of three annual rings, thus forming a band or circle of rot around the central core of wood. This central part of the trunk,  $4\frac{1}{2}$  inches in diameter, was composed of apparently sound wood. Likewise, the area outside the band of rot was macroscopically sound. Because of the relatively large amount of visibly sound wood with reference to the decayed area, the preparation of desirable blocks both for microscopic study and for mechanical tests was facilitated.

Free-hand sections of the wood were made at various distances outside the decayed ring mentioned above. Occasional hyphae were found *one centimeter* out from the noticeable decay and in the apparently sound wood. Here the mycelium was somewhat difficult to detect because of its fine thread-like character and of its extremely hyaline nature. The wood inside the circle of rot was similarly examined. As in the apparently sound wood outside the visible rot, mycelium was found occasionally also in the solid central core. In no part of the macroscopically sound wood, however, were hyphae abundant. Cellulose and lignin tests, made on the wood in which mycelium was found, showed a slight delignifying effect of the fungus.

As in the case of *Polyporus hispidus* on black ash, strength tests were carried out on the oak wood as follows: As many blocks as possible were sawn from the macroscopically sound core of the tree. Although some mycelium had been found in this wood, there were no visible traces of decay in the blocks. The results obtained are recorded in table 8.

TABLE 8. *Summary of Comparative Tests made on Apparently Sound Wood containing Mycelium and on Sound White-oak Wood*

No. of Blocks	Average Area of Blocks (Sq. In.)	Average Failure per Sq. In.
28 apparently sound blocks. ....	1.651	12.624
8 sound blocks. ....	1.112	12.937

Only averages are given. These figures were determined by first finding the failure per square inch of each block and then calculating the average for the blocks used.

Table 8 shows that the average strength of the apparently sound wood is only 313 pounds less than that of the average for sound wood—wood in which no mycelium was found. Such a small difference can not be regarded as showing the true effects produced on the wood by the fungous hyphae. On the other hand, 3 separate macroscopically sound blocks were as much as 3000 to 4000 pounds below this average. Such blocks were undoubtedly weakened by the fungus, as indicated by the tests. There were 18 other blocks, however, that tested slightly above the failure average for sound wood.

### **Fomes Everhartii in Culture**

Three different series of cultures were prepared, namely: on malt-extract agar, on decayed poplar-wood agar, and on decayed poplar wood alone. The latter cultures were mostly studied to determine the effects of the fungus upon the wood. It has already been pointed out in connection with the study of *Polyporus hispidus* that *black lines are formed on the blocks of some wood species and not on others even though they are decayed by the same fungus*. It was this feature which was particularly studied in these cultures on wood.

The mycelium of *Fomes Everhartii* on agar first grows in parallel fibrillose strands radiating from the inoculum. The agar is considerably blackened; in petri-dish cultures this is especially noticeable. In such cultures the mycelium does not continue to grow over the surface of the agar, but appears to be more or less restricted to certain areas. The mycelial growth in such areas becomes dense and is "buff yellow," or in some cases "carob brown" (Ridgway).

In large test-tube cultures, on the other hand, the mycelium discolors the medium just as in the petri dishes, but the growth continues to cover the surface of the agar uniformly. This probably has to do with the amount of moisture present. All the large test tubes had plenty of moisture even 2 months after they were inoculated. Insufficient moisture conditions prevailed in the petri-dish cultures after a few weeks. Certainly neither the lack of food material nor the toxic effect was the controlling factor for the limitation of growth. It is fairly clear that the character of decay in wood depends largely on the amount of moisture available for the fungus. It may be recalled that, during the development of *F. Everhartii* on wood, the rot was localized in some trees to a greater extent than in others. Certainly the darkened zones formed in the wood did not limit the forward growth of the fungus to a marked degree any more than they do in the agar cultures. The greatest rot area in the tree usually occurred near the ground. It may be logically supposed that moisture, aided by other conditions, is the prime factor for the best development of the fungus in the base of the tree.

Can a fungus which is usually found in nature on living oaks continue growth after the tree is dead? Observations in the field have shown that such growth does occur under favorable moisture conditions. Artificial cultures of the wood-inhabiting fungi in the laboratory prove that saprophytic development is possible.

Further cultures were prepared with badly decayed poplar wood placed on malt-extract agar slants in liter flasks (Pl. LIX). The poplar wood was decayed by *Fomes igniarius* and was chosen in order to determine whether the fungus would continue growth after the wood itself had been badly decayed. The mycelium of *F. Everhartii* in culture produced the same effects upon the poplar wood previously decayed by *F. igniarius* as were noted on the agar alone. The wood became discolored and blackened, similarly to the stained zone outside the badly rotted parts of a tree trunk.

Corresponding culture blocks were placed in test tubes containing wet cotton. The rotten poplar wood was inoculated with the mycelium of *F. Everhartii*. No differences were noted between these cultures and those containing agar. The results demonstrate that "wound gum," "protection wood," etc., are not the products of living wood parenchyma, formed to afford protection to the living cells.

*F. igniarius* has been the subject of many investigations because of its importance as a heart-rotting fungus. The studies on it reported here are concerned chiefly with the distribution of the mycelium in the tree.

A beech tree, growing in northern Michigan and infected with this fungus, was selected by forestry students under directions from the writer. The extent of the rot was determined in the field, and the absolute limit for the visible decay was marked by a pencil on the wood section. When the wood slab arrived at Ann Arbor, it was washed several times in sterile distilled water. The slab was then handled as carefully as possible in order to prevent an undue amount of contamination during the isolation of the fungus. The external portions of the wood were first removed with a sterile chisel forceps.<sup>11</sup> Then wood pieces were gouged out from a section of the slab above the visible decay. These pieces were transferred to sterile distilled water, and after several washings were planted in agar tubes.

Although many of the tubes remained sterile, nevertheless, in the course of about two weeks (in some cases longer) several cultures began to show the characteristic mycelium of *Fomes igniarius* (Pl. LX). The successful cultures were obtained from wood pieces taken *two inches* above the limit marked in the field as the extent of the rot. The students were certain that the rot was not visible beyond the marked limit. Here again it was possible to determine the existence of mycelium in what was supposed to be sound wood.

The cultures of *F. igniarius* are very different from those of *F. Everhartii*. The differences are especially noticeable in the growth of the two fungi in petri dishes. Young cultures of *F. igniarius* show a floccose and somewhat tuberculate mycelium in contrast to the radial growth of the parallel and

<sup>11</sup> The chisel forceps used was a modification of the instrument described by E. E. Hubert (Phytopath. **11**: 175. 1921). The tool used was constructed of fine hard steel in such a way as to form a forceps. One end of the forceps was ground down to a cutting edge. The instrument was flamed before using, thus enabling one to gouge out wood pieces from a block and transfer them to sterile agar tubes under aseptic conditions.

fibrillose hyphae of *F. Everhartii*. In addition to the dissimilar growth habits, they are unlike in color. The mat produced by *F. igniarius* is "buckthorn brown" (Ridgway), while that of *F. Everhartii* is "buff yellow" or "carob brown."

From the preceding account it will be seen that *F. Everhartii* and *F. igniarius* may produce similar woodrots, but that the fungi themselves show considerable differences, even if the fruiting bodies and spores are left out of consideration.

#### STUDIES WITH OTHER HEART-ROTTING FUNGI

In order to obtain further evidence of the presence of mycelium in visibly sound wood, it was decided to study the heartrots of still other trees. The fungi and woods studied will be discussed in the order here given: *Hydnum septentrionale* on red maple, *Fomes conchatus* on hawthorn, *F. connatus* on red maple and sour gum.

*Hydnum septentrionale* occurs mostly on beech and maple in the United States. The wood is uniformly decayed throughout the most rotten parts of the trunk and is reduced to a white, soft, and pulpy substance. Radiating lines formed by the more resistant bands of medullary ray cells are characteristic of this rot in maple. These bands of ray cells, in the most rotten wood, may be separated by the fingers in small sheet-like masses. The rotten wood is bordered by a dark-brown-stained zone of firm wood. This discolored wood zone may be several millimeters wide or may be confined within the width of a line. A microscopic examination of wood sections taken from this border zone shows the presence of mycelium. Such discolorations do not limit or separate the sound parts from the badly decayed areas, even though there appears to be an abrupt change from rotten to sound and firm tissues.

In the advanced stages of the rot, the corroded wood fibers and medullary ray cells are practically the only elements left by the action of the fungus. The vessels have almost or completely disappeared. When phloroglucin and hydrochloric acid are applied to such sections, a strong lignin reaction is exhibited by these cells. The presence of some cellulose was also demonstrated in the badly decayed wood by means of zinc chloriodide. In this most rotten wood only a surprisingly few isolated filaments of mycelium could be found. In fact, the disappearance of mycelium in some sections of this wood is almost complete. Since the microscopic examinations were made soon after the tree was brought into the laboratory, the possibility that the hyphae had dried out to such an extent as to make detection impossible does not apply to this case.

Although a microscopic examination of this wood failed to show the presence of mycelium to any extent, inoculations were made on malt-extract agar, using the most rotten pieces of wood. These inocula were selected from various parts of the badly decayed areas. The methods used were similar to those already described for *Fomes igniarius*, except that here



rotten wood was used. No contaminations of foreign fungi occurred, and all the agar tubes remained sterile. It is to be concluded from this result, as well as from the microscopic examination, that mycelium rarely persists in the most rotten wood of red maple decayed by *Hydnum septentrionale*.

*Fomes conchatus* is found on black-ash wood throughout Michigan. In the northern part of the state this fungus is very common on standing trees of this species or on logs in the swamps. In southern Michigan, the fungus is common on black-ash logs, but is rarely seen on living trees. It has been reported on logs of a number of hardwoods (14). My study was made with a partially decayed hawthorn tree infected by *F. conchatus*.

This white rot differs in appearance from that of the apple wood destroyed by *Polyporus hispidus*. *Fomes conchatus* does not destroy the wood elements between the medullary ray cells as uniformly as, and to the extent that, *Polyporus hispidus* does in the apple wood. In general, the rot seems to occur in irregular pockets. This pocket-like appearance of the decay is obvious only in cross sections of the tree trunk. Such decayed areas often coalesce and are not uniformly distributed and defined. The wood between the rotten portions is not discolored, but appears sound to the unaided eye. However, hyphae have been found in microscopic sections made from such wood. Mycelium has been seen in a few of the vessels at a distance of *one centimeter* beyond the noticeably rotten wood.

*Fomes connatus* usually occurs on the species of maple and is a common fungus wherever these trees grow. Occasionally it is found on other trees (14). Apparently it has not previously been known to occur on *Nyssa sylvatica*, a case of which was found and studied for the purpose of making comparison with the same rot produced in maple. The white fruiting body is readily recognized by its distinctly stratified tube layers. Mosses and algae usually cover or partly cover the surface of the fruiting body. These foreign plants on the surface and even in the interior of the fruiting body make it somewhat difficult to obtain cultures from this source.

Two maple trees, *Acer rubrum* and *A. saccharum*, were felled and sectioned for study. The fungus probably occurs more commonly on the latter host in Michigan. The general appearance of the woodrot, however, was the same in both species. The butt logs of both trees were partially hollowed, and both bore the fungous fruiting bodies. In cross section, the rotten wood present was dark brown in color and appeared somewhat stringy. The badly decayed portions were bordered by a brown-stained zone of wood extending out into the light sapwood in finger-like irregularities. The brown and light-colored areas contrasted sharply and represented the discolored and the unaltered wood respectively. Mycelium was found to some extent in the rotten wood and also in the discolored zone. Although a large number of other sections were made of the wood outside the brown border, no mycelium was found here. As far as could be determined in the two maples studied, no mycelium extended into the unaltered wood. It is

entirely possible that such light woods as maple show discolorations more readily than those trees having a naturally darker heartwood, and that even the presence of a few hyphae may soon cause a visible stain.

The rot produced in *Nyssa sylvatica* by *Fomes connatus* is very similar to that already described for maple, and so will not be described in detail. The basal section taken from the tree was hollow like the butt logs of *Acer saccharum* and *A. rubrum*. A discolored brownish zone separated the light sapwood from the distinctly rotten wood.

A microscopic examination for mycelium in the various stages of decay was then made. The sectioning of sour gum was exceedingly difficult because of the hard, tough wood which splits with difficulty. The detection of mycelium in the sections was also complicated by the structure of the wood. Fine, hyaline splints or shreds of cell walls were unusually abundant in all longitudinal or even in cross sections, and could be easily confused with fungous hyphae. This was partly because of the "twisted grain" of this species and partly because the walls of the vessels themselves are very thin. Mycelium was found occasionally, not only in the most rotten wood, but in the discolored or brownish zone as well. Hyphae were also found at a distance of *twenty-seven millimeters* outside the discolored zone. It was only with much patience, however, that mycelium was found in this part of the wood.

The study of this decay was exceedingly interesting, because the rot produced by the fungus was similar in both *Acer* and *Nyssa*, and for other reasons as well. The hosts themselves correspond in many ways, which fact may at least in part account for the similarity of the rot in the two genera. The wood elements of *Nyssa sylvatica*, for example, are uniformly distributed throughout the growth rings and have the general diffuse-porous structure found in maple. In both maple and sour gum the heartwood, especially in young trees, is often difficult to distinguish from the sapwood. It is known (33) that, owing to the large amount of sapwood, *N. sylvatica* is not durable in contact with the soil, and that large trees are often hollow at the butts and sometimes higher up. Whether *Fomes connatus* is the common fungus which causes this decay throughout the area of distribution of this host is not known. It is entirely possible that other fungi may produce a decay of sour gum.

#### DESCRIPTION OF PLATES

##### PLATE LVIII

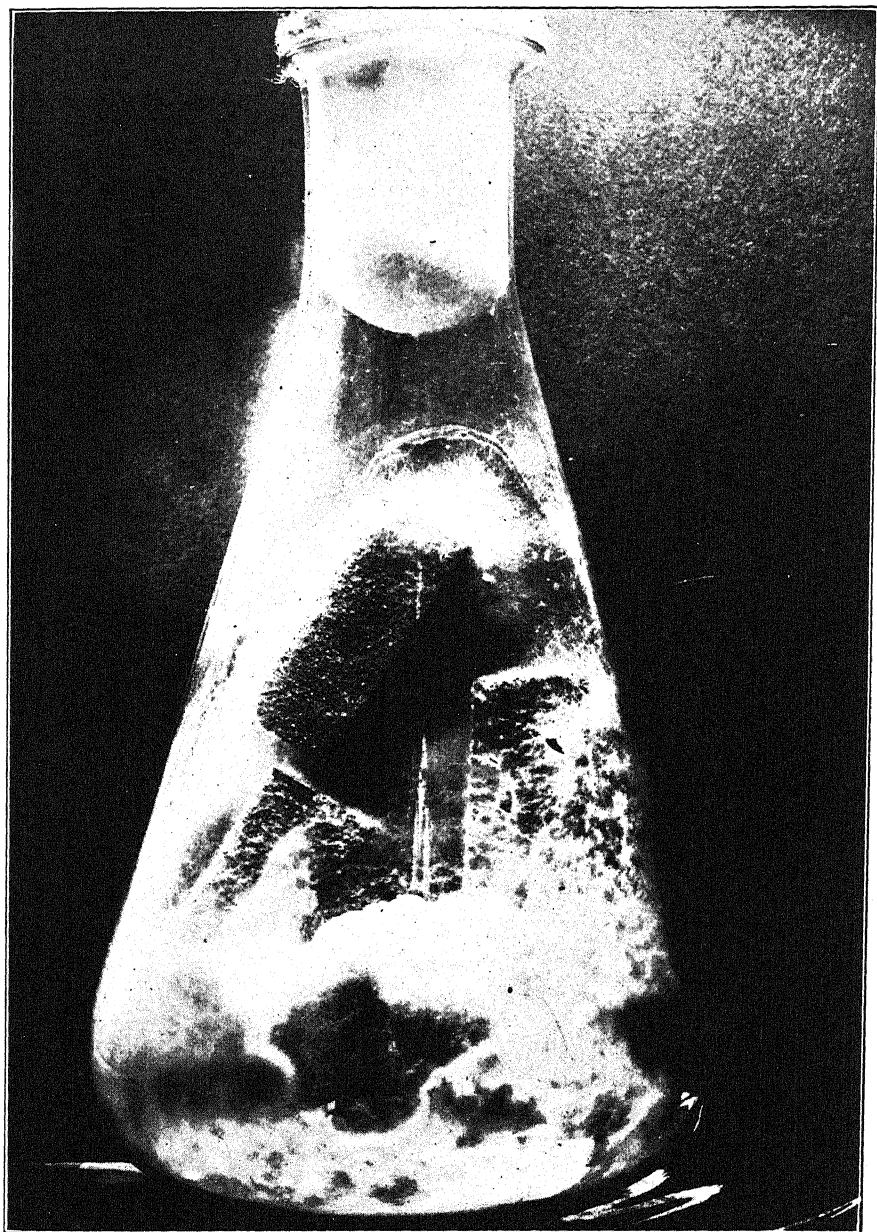
One-year-old culture of *Polyporus hispidus* on red-oak blocks.

##### PLATE LIX

*Fomes Everhartii* grown on blocks which had been decayed by *F. igniarius*.

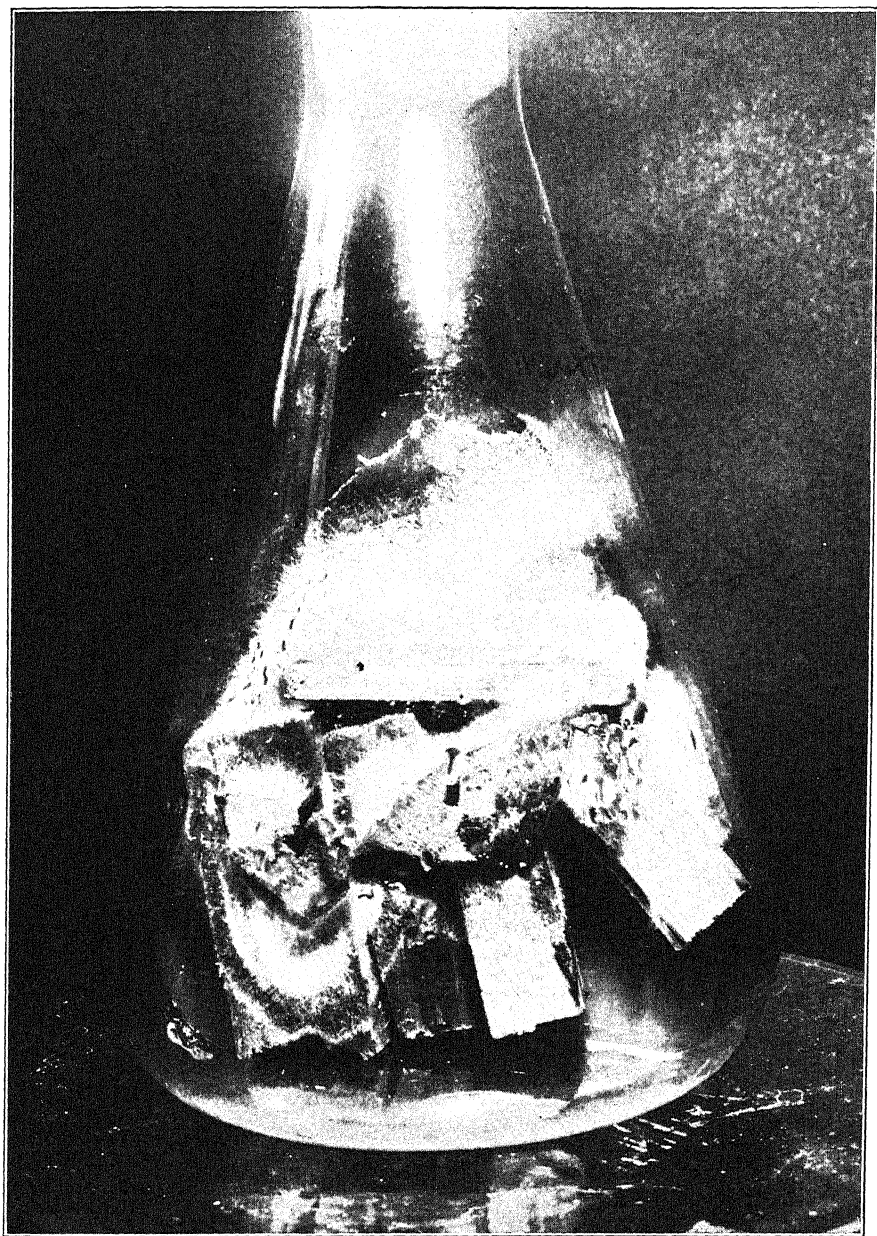
##### PLATE LX

Culture, on the left, of *Fomes igniarius* obtained from the beech slab on the right. The upper part of the slab shows the points at which the wood fragments were gouged out from the apparently sound wood. The culture was obtained two inches above the pencil mark shown in the photograph.



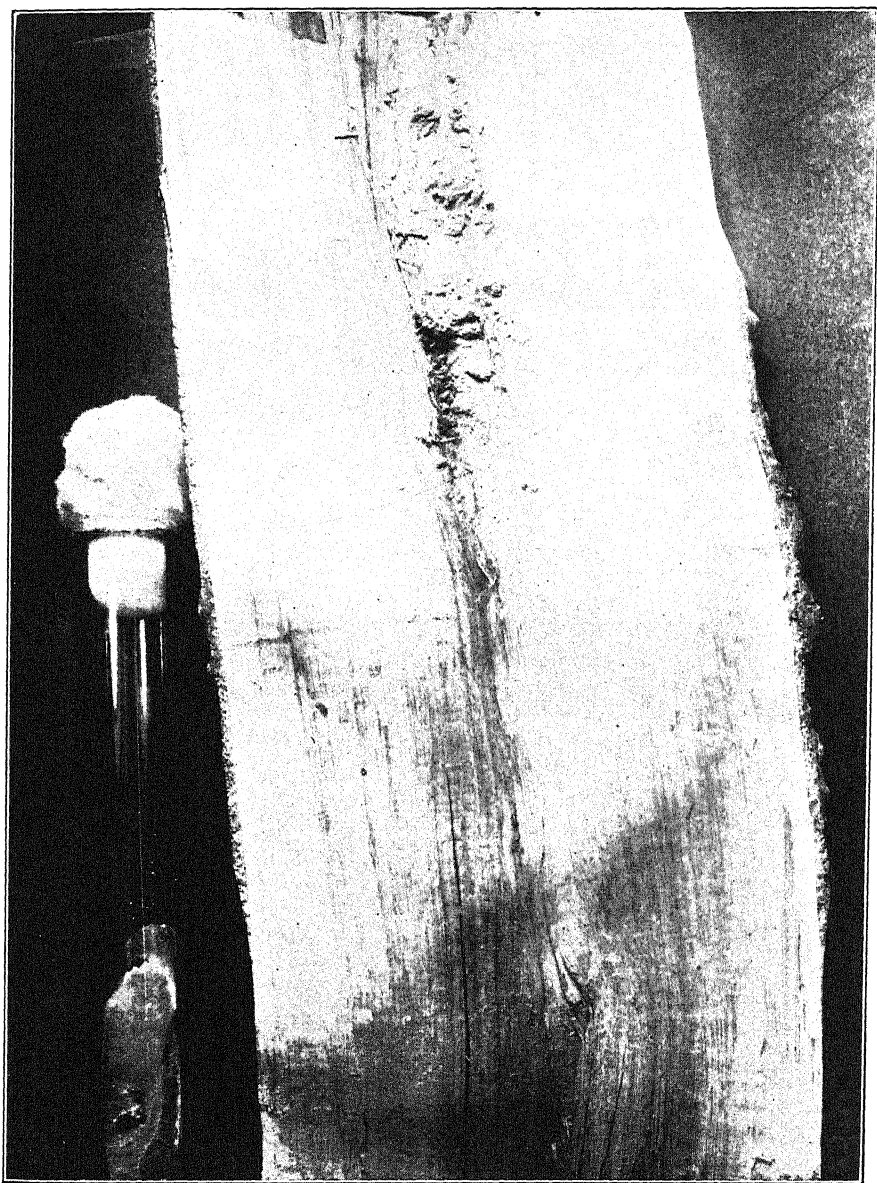
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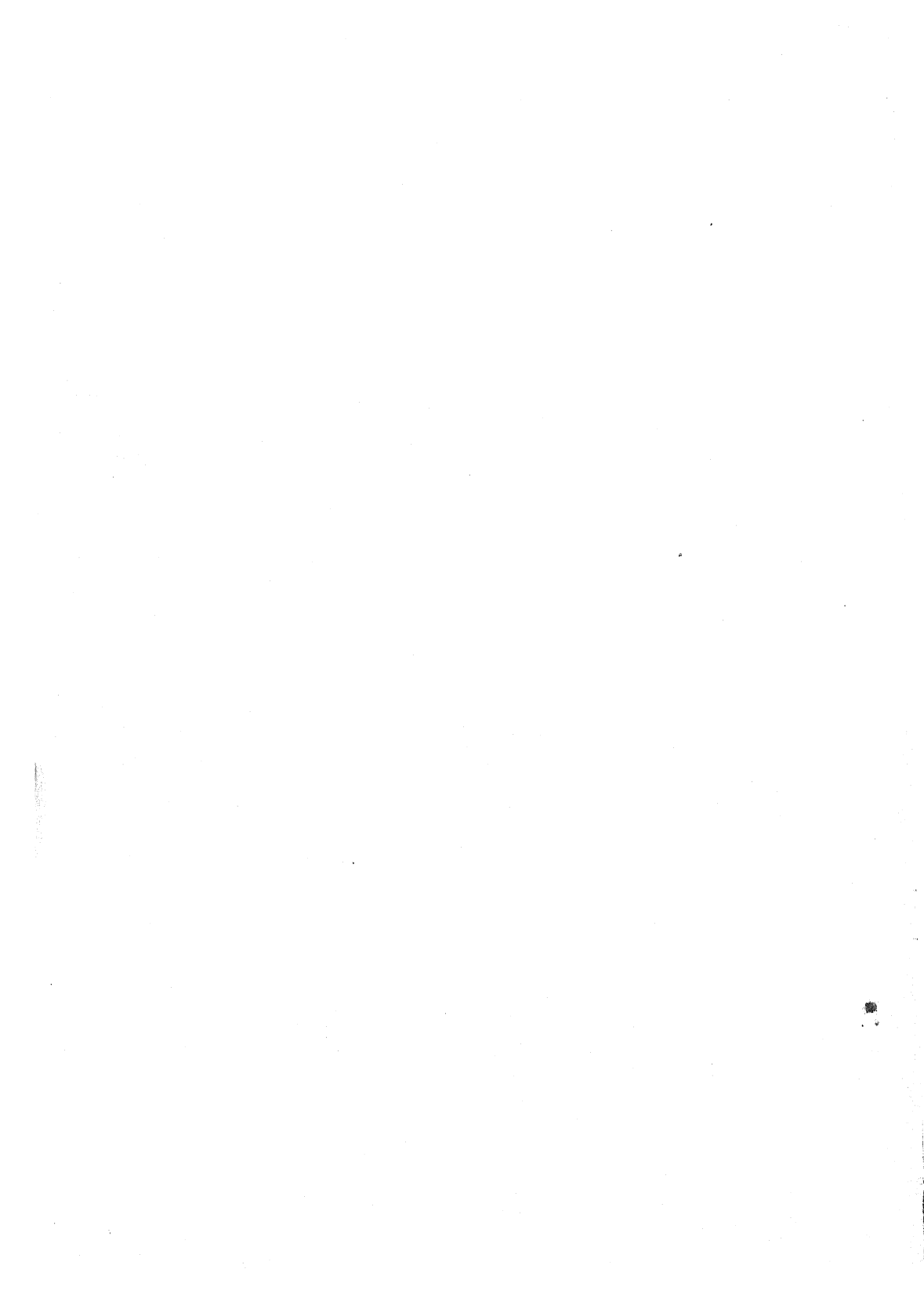


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## THE BIOLOGY AND PATHOLOGY OF SOME OF THE HARDWOOD HEART-ROTTING FUNGI

### PART II

DOW VAWTER BAXTER

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#### STUDIES WITH FOMES "ELLISIANUS" AND FOMES FRAXINOPHILUS

The distribution of a fungus is limited by the combined ranges of its particular host species. It is already known that certain wood-destroying forms like *Fomes officinalis* and *Trametes pini* are in all probability restricted to conifers; while *Fomes Everhartii*, *Fomes rimosus*, and many others are confined to hardwoods. Among the fungi causing decay of frondose trees are a few species which have been found on one or on only a very few hosts. *Fomes fraxinophilus* is a most striking example of this.

*Fomes fraxinophilus*, according to Von Schrenk (30), is found on ninety percent of the standing white-ash trees near the western distribution of the host. Although the fungus is found throughout the entire eastern United States, as far westward as eastern Kansas and Nebraska, it is stated that "it is most abundant where the ash at best is only a tree of medium size and development." Von Schrenk adds: "It would seem that there might be some relation between the greater susceptibility on the part of the ash near its western limit and its generally weaker development at this limit." The fungus has been found on *Fraxinus viridis* as far west as Cooks County, Kansas.

In some field studies of my own concerning the distribution of *Fomes fraxinophilus* I found the fungus on green ash beyond the western limits of the white ash, namely, near Mandan, North Dakota. Here the fungus occurred on this host along the Missouri River flats. It was also abundant along Medicine Creek near Kennebec, South Dakota, where this host was common. A specimen was also collected on green ash near Torrington, Wyoming.

The plant known as *Fomes Ellisianus* Anderson is of special interest in this connection. *F. Ellisianus* was found to be very common along the

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Missouri and Heart Rivers in North Dakota on *Shepherdia argentea*. The same fungus was also found along the North Platte River near Torrington, Wyoming. Murrill (23) records it also from Montana, Colorado, and New Mexico.

By a study of the fruiting bodies of *Fomes fraxinophilus* on green ash and *F. Ellisianus* on buffalo berry, collected in the same region, it was found that the essential macroscopic and microscopic characters are the same. In addition, the mode of attachment of the fruiting bodies to their hosts corresponds. The pileus and pore surfaces curve outward from the broadly attached basal portions of the body. This makes the fruiting body convex at the surface of attachment, and this convexity is a conspicuous feature when the fruiting body is removed from the tree.

Attention should be called to the presence of the reddish margin in young pilei of *F. Ellisianus*. This color tint is not found in the fruit bodies of *F. fraxinophilus*. Such variations may be readily accounted for by the difference in hosts. A similar variation is found in *F. pinicola* (25). In the case of a hardwood host of the latter fungus, a yellowish color replaces the reddish margin characteristic of the fruiting bodies of *F. pinicola* on conifers. While host distinctions form the basis of separation according to some classifications, the characters just mentioned are not regarded by the writer as of specific rank. Overholts (24) regards *F. Ellisianus* as a distinct plant from *F. fraxinophilus* chiefly because of the margin character of the former plant and the difference in hosts. Lloyd (18), on the other hand, holds *F. Ellisianus* to be a synonym for *F. fraxinophilus*, as he can find no essential differences between the two fruiting bodies.

The field observations made by the writer led to a detailed study of these two plants. Laboratory cultures were made in order to obtain further information regarding the mycelial growth of these two fungi on ash and buffalo berry, to make comparisons of the rots produced, and to determine if possible the identity or otherwise of the two fungi by culture methods.

#### Extent and Description of Visible Decay

The wood of *Shepherdia argentea* consists of very sharply defined heart- and sapwood. The sapwood is normally four or five rings in width and is pale yellow in freshly sawn wood. Upon exposure this color darkens into deep lemon yellow in contrast to the cinnamon-colored heartwood.

The rays are of the uniseriate and biseriate type, the latter being the more prevalent and most commonly four to seven cells wide. The numerous vessels have simple end-wall perforations. The walls are marked with bordered pits. The wood fibers are thick-walled cells with large lumina and oblique slit-like simple-bordered pits.

The fruiting bodies of the fungus and the dead tops of the trees were the chief outward indications of the diseased wood in the field. Although the fruiting bodies of *Fomes Ellisianus* usually occurred on the main trunks

or on older branches, one or two feet from the ground, they were occasionally found two thirds up the height of the tree. The limbs bearing the fruiting bodies were either dead or in a dying condition. Those parts of the tree free from the fruiting bodies, on the other hand, usually showed few or no outward signs of decay. Sections made from such limbs, however, were examined microscopically, and abundant mycelium was seen throughout the wood. Lack of moisture, in all probability, restricted fruiting of the fungus, and therefore the tree can not be judged as sound solely by the absence of fruiting bodies. The writer has observed many such cases in lodgepole pine stands badly infected with *Trametes pini*. Because of the dry sites on which *Pinus contorta* was growing, no fruiting bodies of the fungus developed in spite of the large amount of rot present in the tree.

Trees infected with *Fomes Ellisianus* usually ranged from 2 to 3½ inches in diameter. Ring counts showed them to be about 20 years old, although undoubtedly the rot occurs in trees much younger. Like most of the heart-rotting fungi, the organism gains entrance through wounds and finally extends through the entire tree, attacking the small branches and even the roots of the tree. Mycelium has been found in roots only one half inch in diameter. It is likely that infection will occur if such roots come in contact with wounded roots of other buffalo berries in the same clump. As it was impossible to carry on infection experiments of this nature in the field, this latter point was not experimentally determined.

The color of the infected heartwood is changed to a brown color, slightly lighter than the cinnamon color of the normal wood. The sapwood is considerably darkened, and in badly decayed sections can not be distinguished from the heartwood.

Small irregular whitish flecks are scattered throughout the rotten wood. These flecks, which are in reality small areas in which the wood is dissolved or partly dissolved, produce a characteristic mottled effect upon the wood. Such an effect readily distinguishes this rot from the decay produced in white ash by *Fomes fraxinophilus*. The rotten wood of both hosts, however, remains intact, *i.e.*, the trees do not become hollow. The absence of definite black or dark lines in the decayed wood is also common to both rots. However, a slightly darkened zone may appear at times in white ash infected with *F. fraxinophilus*.

The hyaline mycelium of *F. Ellisianus* spreads throughout the wood substance, including both heart- and sapwood. Unlike white ash attacked by *F. fraxinophilus*, the infected portion retains its woody character. The mycelium does not destroy the wood uniformly, but breaks down small areas already referred to as flecks. These broken-down areas are poorly defined and not clearly limited. Mycelium ramifies throughout the elements and is not localized within these areas. However, microscopic mycelial pockets may appear in a radial section of the decayed wood. In such microscopic pockets, the mycelium fills the dissolved or partly dissolved vessels and the surrounding broken-down cells.

The walls of the cells surrounding these pockets remain lignified, as shown by lignin tests. The wood fibers in some sections show a faint blue when treated with zinc chloriodid. The badly corroded medullary ray cells for the most part remain brown when treated with this reagent. A summary (table 9) of the lignin and cellulose tests made on both the decayed heartwood and the sapwood shows the reactions obtained.

TABLE 9. *Results of Staining for Cellulose and Lignin in Badly Decayed Wood of Shepherdia argentea*

Cells	Lignin Test	Cellulose Test
Wood fibers.....	A slight lignin reaction	Very slight reactions in some.
Medullary rays.....	A slight lignin reaction	Cells remained brown in most sections.
Vessels.....	Definite lignin reaction	Negative test in most sections. A slight blue, however, was noted in some vessels.

There are, of course, some differences between the rots produced in the ash and in the buffalo berry, but such variations are to be expected when one considers the dissimilarity of the hosts and their histological structure. On the other hand, the rots produced resemble each other in that there are no definite black lines visible in cross sections of decayed trunks; the decayed wood remains intact, *i.e.*, the trees do not become hollow, and both the heartwood and the sapwood of the hosts are attacked. Although the effects produced on wood by a fungus are sometimes considered useful for the classification of the fungus (in *Fomes putearius* Weir, for example), certainly the characteristics of the rot should not be relied upon too strongly in taxonomic work. However, the growth habits of a fungus have been only too often underestimated. (See footnote, page 527.)

#### **Fomes fraxinophilus and Fomes fraxinophilus forma Ellisianus in Culture**

In order to study the living mycelia of the two fungi under discussion, 36 large test-tube cultures were prepared. The fungi were isolated, one from fruiting bodies produced on *Shepherdia argentea* and the other from fruiting bodies found on *Fraxinus americana*. The mycelia were grown in two series on three different substrata: cornmeal, carrot, and malt-extract agars. One set of cultures was placed in the dark and the other in such a position that light fell directly on top of and parallel to the vertical tube. Opaque paper was placed around the cultures so that no light entered from any other direction. While slight variations appeared in individual cultures, they were insignificant. Tables 10-13 show the growth characteristics of both fungi.

TABLE 10. *Tube Cultures of Fomes Ellisianus Placed in such Position that Light Fell Parallel to Sides of Tubes*

Medium	15 Days			25 Days			40 Days		
	Color	Texture	Size of Area (mm.)	Color (Ridg.)	Texture	Size of Area (mm.)	Color (Ridg.)	Texture	Size of Area (mm.)
Corn-meal	White	Arachnoid	10 x 17	White	Arachnoid-granular	12 x 72	White to massicot yellow	Arachnoid-granular	Test tube filled
Carrot	White, slightly yellowish	Arachnoid-fibrillose	10 x 15	White, ivory yellow	Densely interwoven fibrillose	12 x 87	White to pinkish buff	Densely interwoven fibrillose	Test tube filled
Malt extract	White, slightly yellowish	Arachnoid-fibrillose	11 x 24	White, ivory yellow to pinkish buff	Fibrillose, pores forming	12 x 87	White to chamois cream buff	Fibrillose, pores formed	Test tube filled

TABLE 11. *Tube Cultures of Fomes fraxinophilus Placed in such Position that Light Fell Parallel to Sides of Tubes*

Corn-meal	White	Appressed to arachnoid, pores forming	11 x 9	White to light buff	Fibrillose to densely interwoven fibrillose, pores forming	11 x 61	White to cartridge buff	Fibrillose to densely interwoven fibrillose	Test tube filled
Carrot	White	Appressed to arachnoid	5 x 8	White to light buff	Densely interwoven fibrillose, pores forming	Test tube filled	White to cartridge buff	Fibrillose to densely interwoven fibrillose	Test tube filled
Malt extract	White	Appressed-arachnoid, pores forming	9 x 7	White to light buff	Densely interwoven fibrillose, pores forming	Test tube filled	White to cartridge buff	Densely interwoven fibrillose	Test tube filled

TABLE 12. *Tube Cultures of Fomes Ellisianus Placed in the Dark*

Medium	15 Days			25 Days			40 Days		
	Color	Texture	Size of Area (mm.)	Color (Ridg.)	Texture	Size of Area (mm.)	Color (Ridg.)	Texture	Size of Area (mm.)
Corn-meal	White	Arachnoid	8 x 15	White	Fibrillose, pores formed	Test tube filled	White to cream buff	Fibrillose to densely interwoven fibrillose, pores formed	Test tube filled
Carrot	White	Arachnoid-fibrillose	9 x 12	White cream color	Fibrillose	Test tube filled	White to cartridge buff	Fibrillose to densely interwoven fibrillose	Test tube filled
Malt extract	White	Fibrillose	12 x 21	White	Fibrillose, pores forming	Test tube filled	White to cartridge buff	Fibrillose, pores formed	Test tube filled

TABLE 13. *Tube Cultures of Fomes fraxinophilus Placed in the Dark*

Corn-meal	White	Appressed-arachnoid	4 x 5	White	Fibrillose to densely interwoven fibrillose	12 x 66	White to cartridge buff	Densely interwoven fibrillose	Test tube filled
Carrot	White	Appressed-arachnoid	5 x 6	White	Fibrillose	Test tube filled	White to cartridge buff	Fibrillose	Test tube filled
Malt extract	White	Appressed-arachnoid	7 x 9	White	Fibrillose to densely interwoven fibrillose, pores forming	12 x 86	White to cartridge buff	Fibrillose to densely interwoven fibrillose, rounded protuberances, pores formed	Test tube filled

Tables 10-13 are self-explanatory. It will be seen at a glance that the two plants grown in the light are very similar. Both fungi showed about the same texture and color characteristics on all three agars and at all three examination periods. The rate of growth of the two forms also corresponded in a marked degree. It is also characteristic of both fungi to form hymenial tubes in cultures (Pl. LXI, A).

There were differences, however, in their mode of growth, great enough to permit identification without observing the labels. The main difference consisted in a more dense growth of mycelium in the case of *F. fraxinophilus*, while that from the buffalo berry was very much more arachnoid in appearance. The hymenophore tubes of the two forms also differed in some respects. Although in one-year-old cultures typical tubes were formed by *F. fraxinophilus* forma *Ellisianus*, in young cultures they had a more "Irpe-like" appearance. The time required for hymenophore- or tube-formation in the latter form was also slightly longer than in the ash fungus, but this feature is not necessarily constant.

The similarities and differences recorded for the cultures in the light corresponded in a general way to those of the series in the dark; however, greater mycelial growth was noted after 25 days in the dark, and tube-formation was delayed. It is interesting to note here that hymenial tubes were formed in both light and darkness. It is possible, however, that a morphogenic or carrying-over effect was produced by the light on the dark-room cultures, during the short time required for examination of the cultures. All the cultures kept in the dark were examined in the diffuse light of the laboratory.

It is thus to be seen that the growth characters of the two plants are largely similar. The variations recorded may readily fall into the category of dissimilarities expected in different physiological strains of a fungus.

Additional cultures of *F. fraxinophilus* were started on pectin agar. Although many fungi produce a maceration of wood in the final stages of decay, few investigations have demonstrated that a wood-rotting fungus is capable of living on pectic substances. The most extensive work of this nature has been carried out by Zeller (38) with *Lenzites saepiaria*. Zeller conducted experiments to determine the macerating power of an enzym dispersion of this fungus on carrot, potato, and beet slices. After 18 hours he noted a maceration effect in the carrot and potato discs.

The rot of *Fomes fraxinophilus* on white ash has been studied by Von Schrenk. He found that, shortly after the fungal hyphae invade the medullary rays and immediately adjoining cells, the middle lamellae disappear.

Ultimately the individual cells become entirely isolated. The wood cells proper are gradually destroyed from within outward, the middle lamellae remaining longest.

My observations on this rot confirm Von Schrenk's studies. In order to support these observations, tubes containing pectin agar were inoculated with *F. fraxinophilus*. While the growth of the fungus was relatively slow, the mycelium covered the agar slants and very definite hymenial tube layers were formed in 3 of the test tubes. No appreciable growth occurred where agar alone served as substratum.

The fact that *F. fraxinophilus* will grow on pectin agar is of special interest, and undoubtedly accounts, in part, for the uniformity of the rot in the badly decayed areas in white ash. It is entirely possible that fungi which are not capable of living on pectic substances are more limited in their growth, hence a different type of woodrot results. Undoubtedly the pectic substances themselves vary in purity or composition in different woods. In such cases a fungus can follow the middle lamellae only with great difficulty. Certainly, both the physiological possibilities of the invading organism and the composition of the host itself must be considered in a study of wood decay.

TABLE 14. *Decay Resistance of White-ash Wood Inoculated with Fomes fraxinophilus*

No. of Block	Oven-dry Weight in Grams before Test	Oven-dry Weight in Grams after 1 Yr.	Loss	Loss %
1	10.802	8.037	2.765	25.5
2	10.549	8.461	2.088	19.7
3	10.633	8.934	1.799	16.9
4	10.759	8.924	1.835	17.0
5	10.820	9.011	1.809	16.7
6	10.609	8.462	2.147	20.2
7	10.247	8.121	2.126	20.7
8	10.587	8.196	2.391	22.5
9	9.436	7.802	1.634	17.3
10	10.907	9.279	1.628	14.9
11	10.629	8.682	1.947	18.3
12	10.560	8.287	2.273	21.5
13	9.446	7.898	1.548	16.3
14	9.092	7.621	1.471	16.1
15	10.682	9.160	1.522	14.2

Average loss %..... 18.5

Average loss % of 5 control blocks..... 2.6

In almost any study of wood-inhabiting fungi, culture methods with the wood are necessary in order to obtain facts concerning the growth of the causal organism under known conditions. In order to compare the growth habits of living mycelium on *Fraxinus americana* and *Shepherdia argentea*, wood cultures of the western fungus and of *Fomes fraxinophilus* were prepared. The methods used for the culture of these two fungi were similar to those already described for *Polyporus hispidus* on wood (Pl. LXI, B). However, all the *Shepherdia argentea* blocks were inoculated on a longitudinal surface. Four series of wood-block cultures were made: (1)



*Fomes fraxinophilus* on *Fraxinus americana*; (2) *Fomes fraxinophilus* forma *Ellisianus* on *Fraxinus americana*; (3) *Fomes fraxinophilus* on *Shepherdia argentea*; (4) *Fomes fraxinophilus* forma *Ellisianus* on *Shepherdia argentea*. The rate of decay produced by these two fungi after the cultures were incubated for one year is shown in tables 14-17.

TABLE 15. *Decay Resistance of White Ash Inoculated with Fomes fraxinophilus forma Ellisianus*

No. of Block	Oven-dry Weight in Grams before Test	Oven-dry Weight in Grams after 1 Yr.	Loss	Loss %
1	10.841	8.546	2.295	21.1
2	11.056	9.230	1.826	16.5
3	9.160	7.374	1.786	19.4
4	9.881	8.115	1.766	17.8
5	9.838	7.915	1.923	19.5
6	9.325	7.392	1.933	20.7
7	10.672	8.783	1.889	17.7
8	10.675	8.778	1.897	17.7
9	10.356	8.718	1.638	15.8
10	10.086	8.125	1.961	19.4
11	9.327	7.460	1.867	20.0
12	10.776	8.893	1.883	17.4
13	9.685	7.622	2.063	21.3
14	9.520	7.590	1.930	20.2
15	11.051	8.717	2.334	21.1

Average loss %..... 19.04

Average loss % of 5 control blocks..... 2.60

TABLE 16. *Decay Resistance of Shepherdia argentea Inoculated with Fomes fraxinophilus*

No. of Block	Oven-dry Weight in Grams before Test	Oven-dry Weight in Grams after 1 Yr.	Loss	Loss %
1	11.005	9.841	1.164	10.5
2	8.756	7.714	1.042	11.8
3	8.430	7.284	1.146	13.5
4	7.042	6.180	0.862	12.2
5	8.760	7.731	1.029	11.7
6	7.888	7.034	0.854	10.8
7	9.468	8.222	1.246	13.1
8	7.495	6.662	0.833	11.1
9	10.483	8.979	1.504	14.3
10	11.274	9.960	1.314	11.6
11	8.921	7.994	0.927	10.3
12	6.305	5.455	0.850	13.4
13	7.867	6.910	0.957	12.1
14	7.589	6.710	0.879	11.5
15	8.169	7.075	1.094	12.1
16	7.245	6.431	0.828	11.4

Average loss %..... 11.9

Average loss % of 4 control blocks..... 0.5

TABLE 17. *Decay Resistance of Shepherdia argentea Inoculated with Fomes fraxinophilus forma Ellisianus*

No. of Block	Oven-dry Weight in Grams before Test	Oven-dry Weight in Grams after 1 Yr.	Loss	Loss %
1	8.964	7.457	1.507	16.8
2	8.697	7.051	1.646	18.9
3	7.483	6.474	1.009	13.4
4	6.760	5.781	0.979	14.4
5	10.024	8.760	1.264	12.6
6	9.735	8.389	1.346	13.8
7	8.363	6.070	2.293	27.4
8	8.463	7.395	1.068	12.6
9	7.717	5.510	2.207	28.5
10	9.058	7.866	1.192	13.1
11	8.879	7.600	1.279	14.4
12	9.622	8.111	1.511	15.7
13	11.435	10.324	1.111	9.7
14	6.642	5.326	1.316	19.7
15	7.381	4.849	2.532	34.3

Average loss % ..... 17.6

Average loss % of 4 control blocks..... 0.5

In a *résumé* of these decay-resistance tests, the results may be expressed as follows:

Blocks of *Shepherdia argentea* inoculated with *Fomes fraxinophilus* show a loss in weight of 11.9 percent; inoculated with *F. fraxinophilus* forma *Ellisianus* show a loss of 17.6 percent.

Blocks of *Fraxinus americana* inoculated with *F. fraxinophilus* show a loss in weight of 18.5 percent; inoculated with *F. fraxinophilus* forma *Ellisianus* show a loss of 19.0 percent.

Attention is called first to the fact that *F. fraxinophilus* does not produce the amount of decay in the wood of *Shepherdia argentea* that is shown by the western fungus. The difference is small, however, and is not nearly as large as that shown by individual blocks of *Shepherdia* inoculated with its own strain. Such individual blocks varied as much as 24.6 percent. On the other hand, all the wood inoculated with *F. fraxinophilus* showed practically the same percentage of loss.

In the case of the white-ash blocks, both fungi produced the same amount of decay, only  $\frac{1}{2}\%$  difference being recorded. These decay-resistance tests furnish additional reasons for considering the fungus found on buffalo berry as only a form of *F. fraxinophilus*.

The growth in the one-year-old cultures on wood resembled the mycelial characteristics already described for the two fungi on agar. The mycelium was very much more dense in the case of *F. fraxinophilus*, and all cultures produced large, rounded mycelial protuberances. Such protuberances were also formed in the wood cultures of the western fungus, but these were not so prominent in size or extent.

STUDIES WITH A FORM OF *FOMES POMACEUS*

## Identity and Technical Description

Typical specimens of *Fomes pomaceus* Pers. were found on cultivated cherry in Nebraska and studied by the writer. Several of these specimens were sent to Mr. C. G. Lloyd, who identified them. A not unusual fungus occurring near Ann Arbor and elsewhere in Michigan shows certain differences from the one found in Nebraska and from other specimens of the true *F. pomaceus*. It was thought for a time that this form in Michigan, which occurs constantly on various species of *Crataegus* and on *Prunus americana*, might be *Fomes fulvus* Fr. A comparison and study of the Friesian description and illustration (*Icones Selectae* Pl. 184, fig. 3) shows that our plant can not be so interpreted.

Since the fungus in question possesses the microscopic features of *F. pomaceus*, and since the habit differences are not considered to be of specific rank, it is here treated as a form only. No account of a similar form or variety has been found in mycological literature. No *Poria* is apparently described to which it could be referred. The differences between the typical plant and the form referred to consist in the constantly resupinate habit of the fruiting body of the latter and in its host relations. It is mostly found on species of *Crataegus* in Michigan, although it is not uncommon also on wild plum in the same region. A specimen growing on a peach tree collected in North Carolina is in the University of Michigan herbarium. The description follows:

*Fomes pomaceus* Pers. forma *Crataegi* f. nov.

Fruiting body growing singly, but often confluent, 4-7 cm. long x 1.5-2.5 cm. wide x .5-1.5 cm. thick, entirely resupinate; margin thick, definite, abrupt, circularly and entirely ridged, obtuse; surface at first "warm buff" to "amber brown" (Ridgway), tomentose; margin becoming glabrous and horny, turning to "hair brown" after wintering; context "cinnamon brown"; tubes "cinnamon brown," 4-6 mm. long, stuffed, indistinctly stratified; pores minute, circular, 5-6 to a millimeter; cystidia lacking; spores hyaline, globose, 5-6  $\mu$  diameter. Gregarious on *Crataegus* spp., *Prunus americana*, and *P. persica*. Frequent. Michigan, North Carolina.

## Extent and Description of Visible Decay

Cross sections of the infected trees show a decided color contrast between the sound or apparently sound wood and that which is noticeably rotten. The normal reddish-brown wood of a healthy trunk is rapidly changed to a much lighter color by the action of the fungus. In outline these areas are irregular, extending outward from the center in a finger-like manner. In some trees, apparently sound areas may remain as small scattered portions completely surrounded by the visible rot. The size of the decayed area increases, and finally both heartwood and sapwood are destroyed. As the

rot progresses radially in all directions, the rather small amount of the spring wood remaining undecayed stands out in the form of slight ridges. This is, in fact, a unique macroscopic character of the effect produced by the fungus on the host. The general characteristics of the rot may also be seen in radial section. If infected wood is split lengthwise with an ax, the sound portions show a relatively smooth surface in contrast with the roughened or uneven appearance of the rotten areas.

The absence of concentric black lines or zones in either the radial or cross section is conspicuous if one has in mind the type of decays produced in trees by such fungi as *Fomes igniarius*. When any sign of a discolored zone does occur, it is very slight.

Both healthy and decayed trees were sectioned. The general action of the fungus on both hawthorn and plum was found to be practically the same, namely, a delignifying effect upon the walls of the elements in both host species. The walls of the wood fibers and of the vessels are the first attacked by the fungus, not only in the heartwood but in the sapwood as well. The walls of the medullary ray cells do not show the pronounced blue color with chlor-zinc-iodid until the very last stages of decay. They may even be quite corroded, appearing only in skeleton form, and still not give a good cellulose test.

#### Extent and Effects of Mycelium

Although this rot is prevalent in southern Michigan, no attention has been paid to the damage done by it. Although the host species may be important for landscape planting, no great importance is to be attached to the rot. Since, however, attention has been directed to the mycelium and its distribution in trunks of hardwoods in general, it seemed worth while to examine this case also. *Crataegus* proved to be excellent working material from the standpoint of the number of trees available for cutting, because of the readiness with which the wood lends itself to the technique used, and also because of the abundance of the rot in the field.

All sections taken from the distinctly rotten wood showed the presence of abundant mycelium. The vessels in cross section were frequently filled with hyphae, and no difficulty was experienced in locating mycelium in the wood cells. The occurrence of abundant mycelium in the most rotten wood is of special interest in view of what had been found in the case of *Polyporus hispidus*. I have pointed out (1) that the presence of mycelium in the most rotten wood decayed by *P. hispidus* may not in all cases be easily demonstrated. With reference to the rot now described, however, the fungal mycelium does not necessarily occur in pockets but is uniformly scattered throughout the decayed areas. Disregarding, for the moment, the various explanations given of what becomes of the mycelium in the most rotten wood of certain other decays, it seems that different wood-rotting fungi act very differently on different hosts. Some decays, apparently, are

produced without a copious mycelial growth of the fungus in the wood. Other fungi, such as this new form of *Fomes pomaceus* for example, produce an abundant mycelium in the wood. Granting optimum conditions for fungus-development in the tree, this species seems to produce more mycelium within the wood than is usually the case.

Various possibilities have been suggested to explain the disappearance of mycelium in the most rotten wood. Hartig reports (9) that

Where the hyphae have to pass through regions of tissue containing no proteids, their apices are supplied with protoplasm which is sent forward from behind at the expense of the older parts of the hyphae. The latter are therefore soon emptied, and become filled with air. Although the empty mycelial hyphae persist for some time, they ultimately disappear under the decomposing influence of the fungus itself. The consequence is that one may frequently fail to find anything of the fungus itself.

Kauffman and Kerber (15) do not attempt to answer the question. The writer has studied many different types of decays especially among the hardwoods, and has found that no general rule holds as to the presence or absence of mycelium. For example, the mycelium of this fungus is found more or less uniformly throughout the decayed wood of *Crataegus*. The mycelium of *Polyporus hispidus*, on the other hand, while occurring here and there in the most rotten wood, usually is found in microscopic pockets. I have noted other such cases, as for example that of *Fomes applanatus* on red mulberry, in which the extremely fine hyaline hyphae can be seen in the freshly sawn wood. The same sections were examined several months later after the wood had dried in the laboratory, and the mycelium was scarcely visible at that time. Mycelium was found, but it was not uniformly distributed throughout the rotten areas. It is thought that drying out affects the fine mycelium in certain rots sufficiently to prevent its detection in the wood. In other specimens of the *Fomes applanatus* rot studied, abundant mycelium was found in the badly decayed sections of apple trunks when freshly cut, and even after they had stood in the laboratory for four months the mycelium was still readily seen. In a rotten core of beech wood, however, that had stood in the laboratory for about eight years mycelium was not common in the most rotten wood. When the hyphae were found, they appeared in more or less definite mycelial pockets. That the effects of drying upon the mycelium prevent the detection of hyphae in very rotten wood is, however, only a suggested possibility. Certainly this is not the case with *Fomes pomaceus* forma *Crataegi* on *Crataegus*. Normal hyphae have been found uniformly distributed throughout the elements of this wood after it had been in the laboratory for over a year.

It has already been pointed out that no black lines limit the decayed area, and that at times the only indication of such is a slightly discolored zone. To determine the extent to which the mycelium had progressed in a radial direction, a microscopic examination of the wood of *Crataegus* was made at various distances from the noticeably rotten wood. Mycelium

was found extending radially for four centimeters in the apparently sound wood of *Crataegus*. Sections from such areas were treated with phloroglucin and HCl and chlor-zinc-iodid. Such tests, when compared with similar tests made on sound wood, plainly showed the first effects of the fungus.

The presence of mycelium five or ten millimeters from the rotten wood is perhaps unimportant for most purposes. In most saw mills the margins left for cull are seldom if ever as small as this. Lumber, too, is graded with reference to the suitability for the use intended, as well as according to the visible defects and the size of the lumber. If the rot produces a noticeable defect, the lumber is classed accordingly and the intelligent lumberman understands the product. However, it is not always possible to determine visibly the extent to which the mycelium extends in the wood, and the grade may misrepresent the actual condition of the plank. The essential point determined with reference to the occurrence of mycelium in macroscopically sound wood is that mycelium does not necessarily produce visible effects upon wood in the initial stages of rot. Furthermore, the actual distance to which the fungus has progressed may vary, depending perhaps upon the fungus, the host species, and conditions of the site on which the tree was growing.

Another factor of importance in this connection is the development of the mycelium in sawn sections. Although there were no marked signs of wood-discoloration visible when the sections were brought into the laboratory from the field, a darkening was later produced. This darkening was due to a slight mycelial development of the fungus which occurred in specimens placed on a damp concrete floor. The brown mycelium from the rotten areas soon developed over the most decayed parts of the wood. The writer has tried in many such cases, as in *Fomes ignarius* on beech for example, to develop mycelium from apparently sound wood by placing the sections on cold damp glass or concrete. All such sections failed to develop basidiomycetous mycelium on the exterior of the wood. The failures may have been due to insufficient moisture.

Methods somewhat similar to those suggested and employed by Hubert (II) were used in order to obtain additional knowledge concerning the mycelium in this wood. Freshly sawn sections of an infected wild plum were brought into the laboratory from the field. A small "bolt" was sawn from the apparently sound wood *one foot* distant from and above the section showing the last visible indications of decay. This piece was washed thoroughly several times in sterile distilled water, and was then split open with a sterile hatchet in such a manner that the bark on one side formed a hinge. The block was opened and one fragment of wood at a time was pried loose from beneath the flap and transferred to sterile agar tubes with a chisel forceps. Successful cultures obtained from the apparently sound wood afford direct proof of the presence of mycelium here. The wood was

sound in all respects to the unaided eye, showing no discolorations or other signs of incipient decay.

The best evidence of the occurrence of mycelium in macroscopically sound wood is a culture obtained from such areas. The fact that cultures fail to develop can not, on the other hand, be considered a proof that the wood is free from mycelium. The time required for hyphae to grow on the outside of the inoculum, the moisture condition, the medium used, and many other factors influence mycelial growth from such wood pieces. Although three cultures were obtained out of a large number of tubes within a relatively short time after the fragments of wood were placed on the agar, others did not develop for several weeks later. Thus the amount of hyphae present in such wood at first appeared to be almost negligible, considering the large number of tubes inoculated. Later, however, other tubes gave additional positive results, indicating that the mycelium was more uniformly distributed in the apparently sound wood than was at first supposed. It is thought that a suitable amount of moisture caused these latter cultures to develop. Had not these wood pieces been first thrown into sterile water, the surface mycelium in the blocks would have dried out. Even in spite of this treatment, drying out probably accounts for the remaining sterile of several cultures. In a few cases probably no mycelium was present.

In working with *Fomes applanatus* on mulberry, the same procedure as above described was followed excepting that the sterile-water process was omitted. The mulberry wood was first examined microscopically in order to determine whether hyphae were present in areas apparently sound. After some difficulty, due to the very fine hyaline nature of the mycelium in such wood, fungus hyphae were located *five centimeters from the noticeable rot*.

Where there were no visible indications of rot, wood pieces were removed under aseptic conditions to agar tubes. These fragments were taken from the same portions of the blocks in which mycelium had been found. No cultures of *F. applanatus* mycelium were secured. This is chiefly accounted for by the fact that the mycelium in the wood fragments had possibly dried out and was not longer able to rejuvenate under these conditions.

It is of interest here to note that no black lines were present limiting the decayed areas in the mulberry trunk. Black lines are usually very characteristic of decay in other hosts where *F. applanatus* is present. Heald, in describing the rot caused by this fungus on rotten wood, states that black lines run in a general transverse direction in the wood. White, however, was unable to verify Heald's observation on this point. He finds that "black lines are encountered, but cultural or other evidence shows that when they do occur more than one species of fungus is at work."

### **Fomes pomaceus forma Crataegi in Culture**

The cultures obtained from the apparently sound wood show the identical color and growth characteristics displayed by those obtained from rotten wood or from fruiting bodies. For further comparisons of this nature, isolations of the fungus were also made from both infected hawthorn and wild plum trunks. The difference in hosts from which the inocula were obtained seems not to affect the cultural characteristics of the mycelium. There were no appreciable differences between cultures obtained from decayed wood and those secured from fruiting bodies developed on the two hosts. Subcultures were made, but no growth differences were noted between these and the original cultures. These observations agree with those of Long and Harsch (19), who secured initial cultures from 9 strains of *Trametes pini*, 5 from the tissues of fruiting bodies, and 4 from infected wood. Seven hosts were represented by these cultures. No appreciable differences between cultures obtained from wood and those from sporophores were found. These authors also noted that taking inocula from the different hosts of the fungus resulted in no marked differences in the fundamental cultural characters.

The mycelium of *Fomes pomaceus* forma *Crataegi* was grown on malt-extract agar. At first the mycelium appears whitish. It soon, however, turns to "maize yellow" (Ridgway), later becoming "cinnamon brown." In young cultures the mycelium is somewhat floccose, but later the cultures become lacunose in appearance. In cultures kept for over 18 months the color darkens to "snuff brown" (Ridgway), but the other characteristics mentioned persist.

No attempts were made in the laboratory to obtain fruiting bodies of the fungus. No pore surfaces formed even after cultures had been kept growing for over one and one half years.

### DISCUSSION

A comparative study of a number of heart-rotting fungi and of their effects in the living trees and on sterilized wood makes it possible to look for general correlations in the reactions between fungus and wood. The vital activities of both the host plant and the fungus are dependent upon several factors, such as the complex inherent characters of the organism itself, the appropriate available food supply, and various other environmental influences. The best development of the fungus takes place when all the most favorable growth conditions exist at the same time. Likewise, the tree shows the best growth when all the site conditions for it are optimum. But a great complexity of interrelations arises between the fungus and the host, since the metabolism of each is influenced by the activities of the other. Consequently, it is important to study comparatively the various heart-rotting fungi and their effects upon wood.



The characteristic effects produced in the heart-decay of trees by one species of a fungus do not necessarily correspond to those produced on the same wood by a different fungus. For example, *Fomes connatus* on maple "hollows" the tree and leaves the remaining rotten wood substance in a somewhat stringy condition. The wood of the same species of maple, *Acer rubrum*, on the other hand, is reduced by *Hydnum septentrionale* to a soft mass capable of being separated in thin, plate-like sheets.

On the other hand, different wood species do not necessarily show different effects when attacked by the same fungus. An example of this is the fact that either *Fomes Everhartii* or *F. igniarius* may show similar decays on different hosts as well as on the same host. However, even though the decays produced in wood are similar in these cases, it was shown that these two fungi in themselves are very distinct in their growth habits on artificial media.

Another relation was found in the wood cultures of *Polyporus hispidus*. In this case, the same fungus produced different decays on different woods. On apple blocks definite dark lines were produced, and the rays were left as prominent laminations. Such rays are inconspicuous in sound wood. The decayed blocks, too, were whiter than the sound apple wood. In the blocks of the red oak, no appreciable color changes were produced in the wood. No black lines appeared on the wood cultures, and the rot was characterized by an enlargement of the large vessels.

These changes involve not only the activities exhibited by the fungus, but also a consideration of the wood structure of the tree. That the character of the rot produced depends somewhat upon the histological structure of the tree is shown in the case of *Fomes fraxinophilus* and *Polyporus hispidus* on the ashes. The field observations on the former showed that white-ash wood splits in concentric shells when decayed by this fungus. Black-ash wood shows the same characteristics when decayed by *Polyporus hispidus*. When the most rotten wood of *Fraxinus nigra* is placed on the Riehle machine and compressed, the wood splits in rings. Sound ash wood does not show this kind of a failure under compression. An examination of the structure of the ash wood will show the reasons for this behavior.

Ash is a ring-porous wood which shows a tendency towards brashness (brittleness). The springwood is composed of large vessels; in the summer wood the vessels are small and more widely separated. Forsaith (6) says that "such a restriction of the large conducting elements to one region creates a zone of weakness." He also points out that, in the case of suppressed trees, the spring vessels may occupy over two thirds of the area of the year's increment. When trees are so constructed, it can be more readily understood why the wood, when weakened by the action of a fungus, may split concentrically along these differential planes. It is apparent, therefore, that one can not identify with certainty the causal fungus of a rot solely from the manner in which it affects the wood.

Black boundary lines or zones have been long discussed with reference to wood-decay, and many tentative explanations have been given for their occurrence. There have been few experiments (none in this country) conducted to test such hypotheses. Frank (7) held that the discolorations are the product of living cells. In a study of *Fomes nigricans* on birch, Lindroth (17) concluded that such products furnish a protection to the unaltered wood. But the occurrence of black lines on culture blocks can not very well be accounted for by the activities of living cells. It will be remembered that the blocks of heartwood in these cultures were first dried to constant weight and then sterilized in an autoclave. Münch (22) considers that such discolorations may be formed by dead cells. However, these inclusions usually accumulate where dying of the cells is more or less gradual. According to Rhoads (27), Münch says that "the wood cells have the maximum time to attract material to them before they in turn become attacked and killed by the fungal secretions or other injurious influences." To show that partially live or dying tissue is not a condition for the formation of black lines, poplar blocks, already much decayed by *Fomes igniarius*, were steam-sterilized and then inoculated with *F. Everhartii*. Black zones were readily produced in various regions of the blocks. Furthermore, in cultures of *Polyporus hispidus* in the presence of wood, black or dark-brownish irregular lines were formed on the agar. These lines were conspicuous when the mycelial mats were removed from the flasks. The agar was not discolored in the manner in which certain species of *Fusarium* darken an agar medium. Instead, there were sharp, definite lines corresponding in every respect to those obtained on the wood blocks in culture and similar to those accompanying certain wood rots in nature. After the cultures of *Polyporus hispidus* on the various hardwood species had been growing for one year, irregular black lines appeared on the white-ash, black-ash, yellow-birch, and apple blocks. That these lines are fundamentally products of decomposition can not be doubted. *While these lines were also formed in agar cultures, they appeared only in cultures containing wood blocks.* In sawdust-agar cultures a darkened zone surrounded the mycelium which in no respect, excepting color, resembled the distinct lines. There is evidently a decomposition going on in the wood, the product of which is deposited along localized areas in consequence of some relation between the fungus and the substratum. This is shown rather conclusively by the fact that no dark lines are formed on oak blocks with *Polyporus hispidus*, while they are formed on all other wood species tested with this fungus. Certain woods apparently show discolorations so regularly that the discolorations become almost a characteristic property for that species of wood. In such cases, however, casual observations may fail to locate some other fungus capable of producing these particular effects.

The rate of decay of wood as shown by decay-resistance tests also points to a definite relationship between the properties of the fungus and of the

wood—more strikingly in some cases than in others. Tables 2-6 show that the same fungus is not capable of producing the same amount of decay in different woods. The degree of decay produced in white-ash wood, as shown by the loss in weight, was 3.3% greater than in apple wood; in yellow birch the amount of decay was 13.9% greater than in red oak. On the other hand, both black and white ash show about the same resistance to the attacks of *Polyporus hispidus*. Of equal importance is the fact that white ash showed approximately the same amount of decay regardless of the particular heart-rotting organism. The loss in weight of this wood when decayed by *Polyporus hispidus* during one year was 19.2%; by *Fomes fraxinophilus* forma *Ellisianus*, 19%; and by *F. fraxinophilus*, 18.5%. Such tests indicate that specific changes in a wood tissue due to decay may depend first upon the species of wood attacked, second on the definite wood-rotting fungus, and finally upon a combination of wood and fungus in addition to environmental factors.

The presence and effects of mycelium in wood are often difficult to demonstrate. Mechanical tests of oak decayed by *Fomes Everhartii* showed an average difference of only 113 pounds per square inch shown between the failures of the sound and of the apparently sound wood respectively. The *a priori* idea that the mycelium of a wood-inhabiting fungus reduces the strength of wood apparently does not always apply. In addition to the variables of wood alone, certain fungal mycelium seems to have a different effect upon the cells. Von Schrenk (31) determined the average crushing strength and the average cross-breaking strength of western yellow-pine timber infected with the blue-stain fungus, *Ceratostomella*. His figures show that the "blue" wood is actually stronger when compressed both crosswise and endwise. He points out that, under the conditions existing in the Black Hills Forest, the "blue" timber is certainly very much stronger than the uninfected wood.

The progress of the visible decay supplies an interesting problem and one not easily solved. It would appear that the advance at the periphery of a rot depends upon the distribution of the food material in the bole of the tree, and upon the ability of the fungus to utilize that food. If this food is in a stored condition, let us say in the medullary rays, one would expect the rot areas to increase in the direction of the rays. The studies made with *Fomes Everhartii* and other heart-rotting fungi, however, point to the fact that the decay generally advances more rapidly in a linear direction. That is to say, the visible rot extends farther in a vertical line before the entire diameter of the tree trunk is attacked by the fungus. Using *F. Everhartii* again as an example, the rot may advance in the form of two cones, their bases meeting at the point of greatest decay; or some trees may be completely infected before any one part of the wood is entirely disintegrated. In one of the oaks decayed by *F. Everhartii*, the visible rot had not advanced beyond a small part of the infected trunk. In this

area, however, the wood was badly decayed. In other oaks the noticeable rot occurred throughout the main trunk but the wood was not yet appreciably destroyed. In some trees the rot areas appeared to be well defined, while in others there was more of a gradation between the sound and the noticeably decayed wood. Von Schrenk has found in the case of *Polystictus versicolor* on lilac, for example, that "the line between sound and decayed wood is so sharp that entirely decayed fibers adjoin perfectly sound ones."

That mycelia of heart-rotting fungi may produce no macroscopic evidences of rot in the initial stages of decay has been shown by Kauffman and Kerber (15). In my studies, this fact was demonstrated in the following cases: in black and in white ash affected by *Polyporus hispidus*; in oak attacked by *Fomes Everhartii*; in beech wood affected by *F. igniarius*; in *Nyssa sylvatica* infected by *Fomes connatus*; in *Crataegus* wood infected by *Fomes pomaceus* forma *Crataegi*, and in others.

The question as to what causes hyphae to persist or disappear in the most rotten wood has not yet been answered. I have already suggested that drying effects may account for some of the difficulties encountered in detecting hyphae in wood. *Polyporus hispidus*, either on wood lying on agar or on wood alone, is characterized by the size and abundance of the hyphae in the cultures. One year after the wood was inoculated, the badly decayed blocks of ash and other wood used were examined for fungal hyphae. Abundant mycelium was found throughout the elements in the interior of the blocks. This mycelium was apparently well nourished, judging by the large fungal threads. Swollen parts of the hyphae in the medullary ray cells of black ash measured seven microns in width! The abundance of this mycelium in the very rotten wood shows that the fungus had not suffered from a lack of food material. In other words, the absence of mycelium in badly decayed black-ash wood in nature can not be wholly accounted for by inadequate nourishment. Furthermore, the decomposing wood cells evidently produced no toxic substances which appreciably injured the mycelium in the culture blocks. Why then should toxic substances produced from wood be used to explain the absence of the hyphae in many areas of the most rotten wood? Toxic substances certainly produced no appreciable injurious effects upon the mycelium in these culture blocks during one year. Moisture is apparently a much more important factor and probably accounts for the presence of mycelium in such rotten wood of black ash; at least, optimum moisture conditions were present throughout these cultures of *Polyporus hispidus*.

A heart-rotting fungus such as *P. hispidus* often exhibits both saprophytic and parasitic growth potentialities. The cultures just mentioned demonstrate that this fungus is capable of saprophytic growth on sterilized black ash, white ash, apple wood, and yellow birch blocks. The decay of black-ash wood in these cultures corresponded to that produced in the

heartwood of standing trees. Mycelium of this same fungus obtained from cultures grown in the laboratory was used to inoculate living white-ash trees. Growing black-ash sprouts containing no heartwood were also artificially and successfully infected with this fungus. The inoculation experiments, together with the studies made on decayed sapwood naturally infected, show the parasitic nature of *Polyporus hispidus*. Such examples of facultative parasitism, however, are not limited to the true heart-rotting fungi. It has been found (32) that *Polystictus versicolor* is wholly confined to the sapwood in such trees as oaks, black walnuts, and red gum, in which the sapwood is sharply differentiated from the heartwood. "In woods where the differentiation between heart and sapwood is indistinct, . . . the fungus brings about the destruction of the sapwood with great rapidity, and even destroys the heartwood."

While it has been shown that black-line formation does not appear to be a response of living cells for protection, it is highly probable that the protoplasm in living cells of wood does interfere with the progress of the mycelium of certain species. In logs, on the other hand, the living protoplasm is probably not present for any considerable length of time so as to prevent fungus-development. The sapwood, containing an abundant amount of food, promotes the development of the fungus in such regions. Hence, the same fungus may bring about the decay of heartwood in the standing tree and continue to thrive on both heart- and sapwood when the tree is felled. It was seen in the discussion of *Fomes Everhartii* that under favorable conditions this fungus may continue to grow on limbs after they have been blown from the tree. In contrast, some wood-rotting fungi show a cessation of growth on the logs after the tree is cut. I have pointed out (1) that no cases have been certainly observed in which *Fomes fraxinophilus* continued growth on white-ash logs. No fruiting bodies of this fungus have been seen on old logs in the field. Certainly fruiting bodies must be very rarely formed on logs. Yet this fungus has been grown successfully on various substrata, including malt-extract and even pectin agars in the laboratory! Likewise, hymenophore tubes were readily formed under artificial conditions. Von Schrenk (29) states that

The mycelium of *Polyporus rimosus* does not grow in the wood of the locust after it is cut from the living tree. Diseased locust wood when used for posts does not continue to rot after it is placed in the ground.

#### SUMMARY

1. The results of this study of ten different heart-rotting fungi demonstrate: (a) that visible changes in tree trunks or in wood generally referred to as "decayed" or in an "incipient" stage of decay can not be used as a criterion of the extent to which the fungus has progressed in the apparently sound wood; (b) that mycelium occurs in a radial or linear direction in advance of such visible decay to a considerable distance, at least several

centimeters in a radial direction and as much as six feet up or down from the visible decay; (c) that mycelium grown on wood in the laboratory does not necessarily produce discolorations or other visible indications of rot in the initial stages of decay.

2. The progress of the mycelium in hardwood trunks affected with heartrot is not limited by the peripheral black lines or discolored zones, even though the decayed areas are visibly bordered by such lines. Therefore, the many discussions concerning the functions of such border zones become inconsequential, and a new explanation must be sought for these border lines.

3. Hyphae are distributed throughout this black line or zone, at least in *Acer rubrum* affected by *Hydnum septentrionale* and in *Nyssa sylvatica* attacked by *Fomes connatus*—the only two cases carefully examined.

4. The presence or absence of mycelium in the rotten core inside the discolored line, or the distribution of the mycelium, does not follow a general rule. The mycelium of *Fomes applanatus*, for example, while occasionally found in the rotten area, can be located only in pockets, and these pockets may often be microscopic or widely scattered. In *Crataegus* sp., however, the mycelium of *Fomes pomaceus* is quite uniformly distributed throughout this area. The other rots studied approach one or the other of these conditions.

5. An improved method was used for the pure culture of wood-rotting fungi. The advantages of this method are: (a) the disposition of the blocks in the flasks as desired; (b) the uniform inoculation of the wood blocks; (c) an actively growing inoculum; and (d) the control of moisture conditions in the flasks.

6. The following six species of wood-destroying fungi were developed on artificial media or on wood blocks in flasks: *Polyporus hispidus*, *Fomes igniarius*, *F. Everhartii*, *F. fraxinophilus*, *F. fraxinophilus* forma *Ellisianus*, and *Fomes pomaceus*. The three latter plants were studied in pure culture for the first time.

7. Each of the wood-rotting fungi cultured on agar produced a luxuriant and distinctive vegetative growth.

8. It is shown that the rate of decay brought about by the same fungus is a factor of the character of the wood, in other words of its decay-resistance, and that this varies with each species of wood. For example, the amount of decay produced in one year by *Polyporus hispidus* on white ash under controlled conditions was 19.2%; on black ash, 17.1%; on yellow birch, 24.4%; on red oak, 10.5%; on apple wood, 15.9%. The rate of decay by one fungus was also shown to be correlated in the different woods by different types of rot, associated in each case with the definite structure of the wood.

9. The field and culture evidence obtained concerning *Fomes Ellisianus* Anderson and *Fomes fraxinophilus* Pk. indicate that they must be con-

sidered as a single species. *Fomes Ellisianus* Anderson is considered a form of *Fomes fraxinophilus* Pk.

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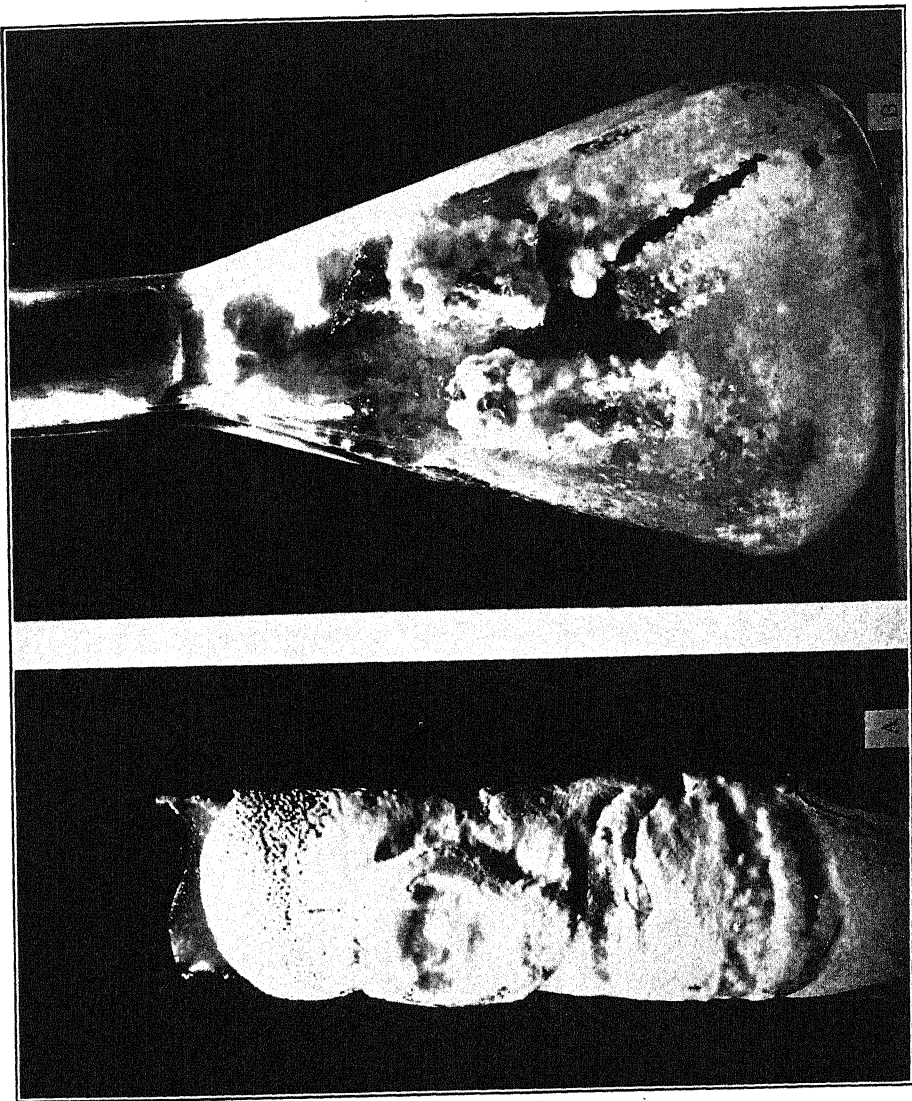
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#### DESCRIPTION OF PLATE LXI

FIG. A. Test-tube culture of *Fomes fraxinophilus*, showing surface (slightly enlarged).

FIG. B. One-year-old culture of *Fomes fraxinophilus* forma *Ellisianus* on white-ash blocks.





BAXTER: HEART-ROTTING FUNGI



# THE ELONGATION OF ROOT HAIRS AS AFFECTED BY LIGHT AND TEMPERATURE

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## INTRODUCTION

The growth of higher plants involves not only cell-enlargement but cell-division and cell-differentiation as well. Cellular interaction may also be a modifying factor. The complexity of the phenomenon of growth is further increased by the fact that each of these phases in turn embraces several distinct processes. Cell-division involves on the one hand division of the plastids, vacuoles, and nuclei, with all the intricacies of mitosis, and on the other the formation of a partition. Cell-enlargement means not only extension of the cell wall and plasma membrane but deposition of new cell-wall material and the absorption of substances, especially foods, salts, and water, either by osmosis or by colloidal imbibition. Finally, cell-differentiation includes thickenings of the wall and changes in its chemical composition, the enlargement and fusion of the vacuoles, the formation of plastids, changes in the shape of the cell, and changes in the accumulation of storage substances, as pigments, foods, and alkaloids. Consequently, no single physical process like colloidal imbibition or hydration can be regarded as fully expressing the phenomenon of growth. It is to be noted that dry-weight increase, which has been frequently used as an index of growth, includes only certain of the processes of growth and also very much which is not growth at all, such as the effect of photosynthesis, respiration, transpiration, etc.

Environmental factors accelerating or retarding growth may accomplish this effect by accelerating or retarding all three phases or any one or two of them. For instance: The rate of increase in size of such a multicellular structure as a root or a stem may be retarded, either by lengthening the time required for cell-enlargement or by lengthening the time required for cell-division. This investigation is an attempt to throw some light upon the solution of this particular question.

Various instruments have been devised for measuring tissue-enlargement, from the simple auxanometer of Sachs (38) to the more complicated instruments of Pfeffer (35), MacDougal (31), Haines (18), Dowling (13), Bose (8), Jones (20), and others. The horizontal microscope has been used in the study of growth in both multicellular tissues and single cells. In the latter case it is necessary to depend almost entirely on this instrument, taking readings at frequent intervals by means of the eyepiece micrometer. It is

obvious that unicellular and filamentous forms of the algae and fungi, where the living cell can be easily isolated and elongation is comparatively rapid, lend themselves best to this method of investigation. Thus Blaauw (5), working on the growth of an individual cell, used the sporangiophore of *Phycomyces nitens*, and Miss Parr (34), in her investigations of the response of an organism to various kinds and intensities of light, has used the sporangiophore of *Pilobolus*. Noll (33) studied growth in *Vaucheria*, paying particular attention, however, to the method of wall-formation. It will be noted that in all three of these cases coenocytic forms have been used, the cells under consideration containing a large number of nuclei in a common cytoplasm. The condition in these cases then may be very different from that in which each nucleus is the center of a protoplast, as is the case in most cells, especially of the higher plants.

Very little if any work has been done on the rate of elongation of uni-nucleate cells of higher plants. A cell to be of value for this purpose must be readily visible with the compound microscope and should be free as far as possible from the influence of cell-division, cell-differentiation, and cellular interaction. That is, it should be a cell which is enlarging but which will not again divide, the differentiation should be very slight, and the cell should be little affected by pressure and tension of neighboring cells. Only a few cells of this kind are to be found in the complex system of tissues such as one finds in the higher plants. Trichomes, pollen tubes, and root hairs are among the few which conform to these requirements. Of these the last named was chosen for this study. In this structure cell-division is completed before the hair begins to form. Although new wall material is doubtless being deposited very rapidly during the growth of the root hair, this is more a matter of cell-enlargement than a case of differentiation, as the term is ordinarily used in relation to cell-growth. Finally, the elongating root hair, as will be shown later, is only for a time directly affected by cellular interaction.

Investigations made on root hairs have been confined to the composition of their walls, the cause of their formation, and the conditions under which they may form or by which their formation may be inhibited. Thus the work of Schwarz (40), Devaux (12), Leavitt (25), Miss Roberts (36), Miss Bardell (3), Miss Snow (44), and others, include investigations on the effect of temperature, transpiration, light, moisture, contact, wounding, mechanical retardation, length of cell, food supply, and oxygen on the initiation or inhibition of root-hair formation. Very little if any work has been done from the standpoint of the normal rate of growth or of the effect of the environmental factors upon the rate of growth.

#### METHODS AND SCOPE OF INVESTIGATION

The pop corn (*Zea mays* L., sub-species *everta* Sturt.), the common garden radish (*Raphanus sativus* L.), and the white mustard (*Sinapis alba* L.), were

selected for this study. Two different schemes of germination were employed. The seeds were first germinated on gauze stretched over glass jars partly filled with water. This resulted in the roots being straight but very slender, and it was later found preferable to germinate the seeds on damp filter paper, laid upon a plate of glass and covered with a bell jar to keep them moist. The whole was kept in the dark at room temperature. By this method more stocky seedlings with a better development of root hairs were secured. Germination began in the case of the radish in about 36 hours, and 12 hours later the roots were of sufficient length to be transferred to the moist chamber described below. The corn was somewhat slower in both germination and growth, about 48 hours elapsing before the pericarp burst.

The moist chambers were a modification of the type used by Blaauw (7) in his work on the growth and phototropism of roots. They were made by using pairs of glass plates, in size 5 x 7.5 cm. These two plates were separated by several thicknesses of filter paper from which two oval sections had previously been cut, leaving the filter paper in the form of a frame. Narrow openings (about 3 cm. wide) were cut at the top, through which the roots of the seedlings were passed. The chambers were bound at the ends with strong linen thread. The filter paper was then saturated with water and the seedlings were inserted (fig. 1, Plate LXII).

The chambers containing seedlings were placed upright inside a large crystallizing dish, covered with a glass, and placed in a dark constant-temperature room. During the earlier trials a thin layer of water was placed in the dish in which the chambers stood. This resulted in moisture condensing on the inner walls of the chambers, which was found to be unsatisfactory because the root hairs so grown were constantly swelling at the tip and bursting, making them useless for study. Miss Roberts (36) found in her investigations that, if moisture was reduced sufficiently, there was a lack of root-hair development or they failed to elongate. She considers that this reduction in moisture content has a direct effect upon the walls, making them less elastic and not so easily extended. It appears then that there is an optimum humidity for root-hair development, below which they do not appear at all and above which they burst. If Miss Roberts' contention is correct, it may be that in a saturated atmosphere the colloidal root-hair wall may be softened by imbibition and be easily stretched to the bursting point under the existing conditions of osmotic pressure maintained in the root hair. It was then found that the proper humidity could be attained by moistening several thicknesses of filter paper and placing them in the bottom of the dish after most of the free water had been removed. The chambers stood erect upon this, and when covered the large container required no addition of moisture for a week or more. Under these conditions, trouble from bursting hairs was nearly eliminated and excellent root-hair development took place. After standing in the constant-temperature room over night, the seedlings were in good condition for use.

The investigations were carried on in a basement room having thick cement walls and no windows. The temperature of this room did not vary more than three degrees during the entire time of experimentation. During the course of any one set of observations covering from three to ten hours, the temperature of the room seldom varied more than one half of one degree. Except in experiments on the effect of light, the only illumination permitted was a red light of low candle power which was turned on only while making the adjustment of the moist chamber on the microscope, and in taking readings. The microscope tube was shifted to the horizontal position so that the moist chamber could be placed on the stage in a vertical position. The mirror was so arranged that the light from the red lamp, placed at some distance at one side, would be reflected in the field of the microscope. A special eyepiece micrometer was used, with an adjusting screw which enabled one to move the micrometer scale across the field of vision at right angles to the length of the scale. This permitted keeping the scale over the root hair during the early part of its growth, when lateral (downward) movement was conspicuous, without moving the moist chamber on the stage.

Measurements were taken of the root hairs every fifteen minutes at first. Later, with a higher-power objective, five- and ten-minute intervals were used. Measurements were begun at the time the root hair appeared as a slight protuberance on the root, and a root hair was always chosen which from its location would be likely to grow at right angles to the axis of the microscope.

#### THE GRAND PERIOD OF ELONGATION OF ROOT HAIRS

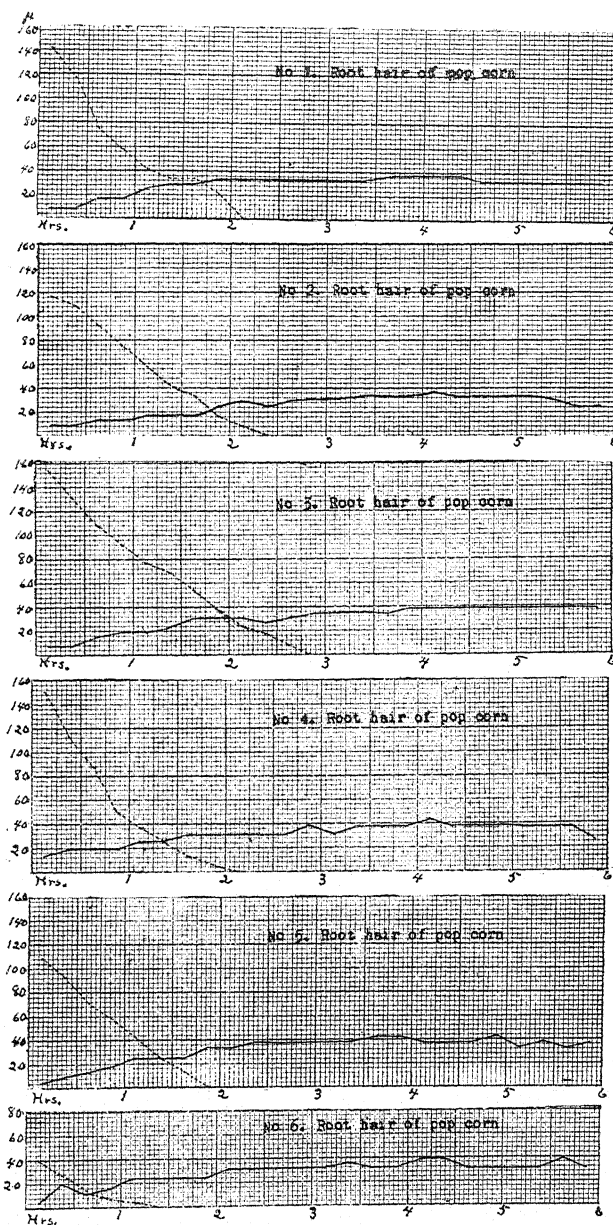
The primary purpose in studying the grand period of growth of root hairs under constant conditions in connection with this investigation was to determine if there is an interval during which elongation is comparatively uniform. If such a period of time exists, then the hair becomes a satisfactory subject for the study of the effect of external factors, such as light and temperature, on the enlargement of the cell.

The lists of readings numbered 1-18 below, and graphs 1-18 (text figs. 1, 2) present the results obtained from the following studies.

(a) Six root hairs of pop corn were measured as to root-hair elongation and lateral movement, readings being taken at 15-minute intervals for six hours, with a view to studying the beginning of the grand period of growth. The temperatures were 23° C. in nos. 1, 2, and 5, and 22° C. in nos. 3, 4, and 6.

Root-hair elongation in microns per 15-minute interval was as follows:

- No. 1. 8.3; 8.3; 16.7; 16.7; 25.0; 29.2; 29.2; 33.4; 33.4; 33.4; 33.4; 33.4; 33.4; 37.5; 37.5; 37.5; 33.4; 33.4; 33.4; 33.4; 33.4; 33.4;  
No. 2. 8.3; 8.3; 12.5; 12.5; 16.7; 16.7; 16.7; 25.0; 25.0; 29.2; 31.3; 31.3; 33.4; 33.4; 33.4; 37.5; 33.4; 33.4; 33.4; 33.4; 31.3; 25.0; 25.0.



TEXT FIG. 1. Six root hairs of pop corn at the beginning of the grand period of growth, showing the relation between root-hair elongation and lateral movement of the entire hair. The solid line gives the root-hair elongation, the broken line lateral movement. Readings taken at 15-minute intervals.

- No. 3. 7.7; 7.7; 15.4; 19.3; 19.3; 23.1; 30.8; 30.8; 30.8; 27.0; 30.8; 34.7; 34.7; 34.7; 34.7; 38.5; 38.5; 38.5; 38.5; 38.5; 38.5; 38.5; 38.5; 38.5.
- No. 4. 12.5; 18.7; 18.7; 18.7; 25.0; 25.0; 31.2; 31.2; 31.2; 31.2; 31.2; 37.5; 31.2; 37.5; 37.5; 37.5; 43.7; 37.5; 37.5; 37.5; 37.5; 37.5; 37.5; 37.5; 25.0.
- No. 5. 4.1; 8.3; 12.5; 16.7; 25.0; 25.0; 25.0; 33.4; 33.4; 37.5; 37.5; 37.5; 37.5; 37.5; 41.7; 41.7; 37.5; 37.5; 37.5; 41.7; 33.4; 37.5; 33.4; 37.5.
- No. 6. 3.9; 19.3; 11.6; 15.4; 23.1; 23.1; 23.1; 23.1; 30.8; 30.8; 30.8; 30.8; 30.8; 34.7; 30.8; 30.8; 38.5; 38.5; 30.8; 30.8; 30.8; 38.5; 30.8.

Lateral movement in microns per 15-minute interval was as follows:

- No. 1. 141.9; 116.9; 75.1; 58.4; 41.7; 33.4; 33.4; 20.8; 00.0 (no lateral movement after 2 hours).
- No. 2. 116.9; 108.5; 91.8; 75.1; 58.4; 41.7; 33.4; 16.5; 8.3; 0.0 (no lateral movement after 2½ hours).
- No. 3. 154.0; 130.9; 107.8; 92.4; 77.0; 69.3; 53.9; 38.5; 23.1; 19.3; 7.7; 0.0 (no lateral movement after 2¾ hours).
- No. 4. 150.0; 112.5; 87.5; 50.0; 37.5; 25.0; 12.5; 6.2; 0.0 (no lateral movement after 2 hours).
- No. 5. 108.5; 91.8; 75.1; 58.4; 41.7; 25.0; 12.5; 00.0 (no lateral movement after 1¾ hours).
- No. 6. 38.5; 27.0; 11.6; 7.7; 3.9; 0.0 (no lateral movement after 1¼ hours).

(b) Six root hairs of radish were measured as to root-hair elongation and lateral movement, readings being taken at 15-minute intervals for from 5½ to 6 hours. The temperatures were 23°–23.5° C. in no. 7, 23.5°–24° C. in nos. 8 and 9, 22.5° C. in no. 10, and 24.5° C. in nos. 11 and 12.

Root-hair elongation in microns per 15-minute interval was as follows:

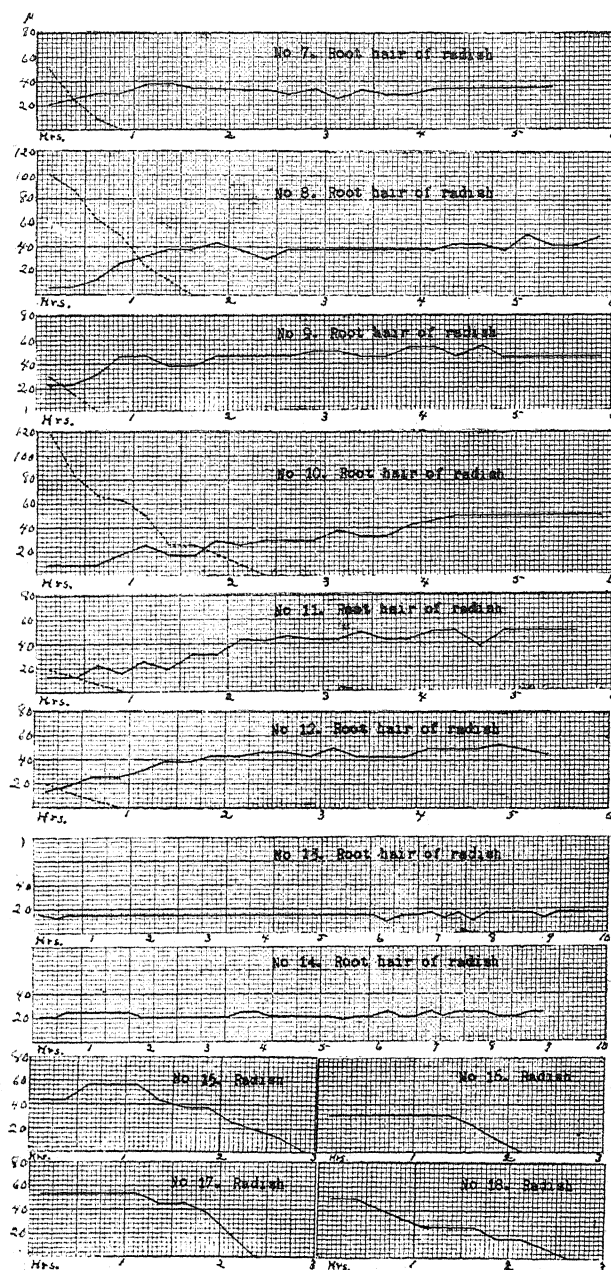
- No. 7. 20.8; 25.0; 29.2; 29.2; 37.5; 37.5; 33.4; 33.4; 33.4; 33.4; 29.2; 33.4; 25.0; 33.4; 29.2; 29.2; 33.4; 33.4; 33.4; 33.4; 33.4; 33.4.
- No. 8. 6.2; 6.2; 12.5; 25.0; 31.2; 37.5; 37.5; 43.7; 37.5; 31.2; 37.5; 37.5; 37.5; 37.5; 37.5; 37.5; 37.5; 43.7; 43.7; 37.5; 50.0; 43.7; 43.7; 50.0.
- No. 9. 23.1; 23.1; 30.8; 46.2; 46.2; 38.5; 38.5; 46.2; 46.2; 46.2; 46.2; 50.0; 50.0; 46.2; 46.2; 53.9; 53.9; 46.2; 53.9; 46.2; 46.2; 46.2; 46.2.
- No. 10. 8.3; 8.3; 8.3; 16.7; 25.0; 16.7; 16.7; 29.2; 25.0; 29.2; 29.2; 29.2; 37.5; 33.4; 33.4; 41.7; 45.9; 50.1; 50.1; 50.1; 50.1; 50.1; 50.1; 50.1; 50.1.
- No. 11. 12.5; 12.5; 22.0; 15.6; 25.0; 18.7; 31.2; 31.2; 43.7; 41.6; 46.8; 43.7; 43.7; 50.0; 43.7; 43.7; 50.0; 50.0; 37.5; 50.0; 50.0; 50.0; 50.0.
- No. 12. 12.5; 18.7; 25.0; 25.0; 31.2; 37.5; 37.5; 43.7; 43.7; 46.8; 46.8; 43.7; 50.0; 43.7; 43.7; 43.7; 50.0; 50.0; 50.0; 56.2; 50.0; 43.7.

Lateral movement in microns per 15-minute interval was as follows:

- No. 7. 50.1; 25.0; 8.3; 0.0 (no lateral movement after 45 minutes).
- No. 8. 100.0; 87.5; 62.5; 50.0; 25.0; 12.5; 00.0 (no lateral movement after 1½ hours).
- No. 9. 30.8; 15.4; 00.0 (no lateral movement after 30 minutes).
- No. 10. 116.9; 83.5; 66.8; 62.6; 50.1; 25.0; 25.0; 16.7; 8.3; 0.0 (no lateral movement after 2½ hours).
- No. 11. 18.7; 12.5; 6.2; 0.0 (no lateral movement after 45 minutes).
- No. 12. 18.7; 12.5; 6.2; 0.0 (no lateral movement after 45 minutes).

(c) Two root hairs of radish were measured as to rate of root-hair elongation after lateral movement had ceased, readings being taken at 15-minute intervals for 10 and 9 hours respectively. The temperature was 23° C. in no. 13 and 24° C. in no. 14.





TEXT FIG. 2. Nos. 7-12 show the relation between root-hair elongation and lateral movement in six radish root hairs at the beginning of the grand period. Nos. 13 and 14 show the rate of elongation of two radish root hairs, studied for 10 and 9 hours respectively, after lateral movement had ceased. Nos. 15-18 show the rate of elongation of four radish root hairs at the close of the grand period of growth. Readings at 15-minute intervals in all cases.



that there may be a relationship between the inability of the cell to elongate vertically and the development of lateral protrusions, namely, root hairs. Indeed, cellular interaction may very probably be an important factor. Miss Snow (44) found in corn roots grown in water, bearing no root hairs, that both epidermal and cortical cells are greatly elongated, while in roots grown in air, bearing root hairs, the cells become shorter and thicker toward the central cylinder. She found experimentally that retarding the vertical elongation of roots, by growing them in small glass tubes closed at the base, resulted in increased production. From this she developed the theory that the retarding effect of the subepidermal cells of the cortex on those of the epidermis initiates root-hair formation. Miss Snow's theory is strongly supported by my data. The root hairs appear on the roots of the pop corn and radish where vertical cell-elongation is being checked, as shown by the decrease in the rate at which the root elongates at this point. Thus the subepidermal cells, checking vertical cell-elongation in the epidermis, bring about the formation of lateral evaginations which later develop into root hairs, an excellent example of cellular interaction.

It is possible also to see how the gradual decrease in vertical elongation of the epidermal cells may account for the gradual increase in the rate of root-hair elongation. A retardation in the rate of vertical elongation of the cells of the root is doubtless accompanied by a gradual increase in the osmotic pressure. If the osmotic pressure continues to increase at the same rate as vertical elongation is retarded, the osmotic pressure of the cells will increase enormously. Now, in the subepidermal cells of the cortex this increased pressure would be equalized by the pressure of the adjoining cells on opposite sides of the walls between them. The same would be true of the lateral and inner walls of the epidermis. Miss Roberts (36) found a pressure equivalent to five atmospheres in the epidermal cells which she studied. This means that the outer wall of the epidermal cell is under strong pressure from within, five times as great as that on the outside. As the pressure here becomes gradually greater, the outer wall tends to push out. If a portion of this wall is weaker than the rest, it would be extended, forming a narrow protuberance. Such may be the explanation of the first appearance of the root hair. Its rate of growth should gradually increase as the pressure in the cell increases. When lateral movement has ceased, usually within two to three hours or less in the roots studied, then the internal pressure in the cell becomes stabilized and elongation of the root hair continues, if kept under uniform conditions, with only minor variations in its rate of growth.

There is some difference as regards the length of time lateral movement continues in the case of different root hairs. Thus for the corn the shortest time was  $1\frac{1}{2}$  hours and the longest 3 hours, with an average time of  $2\frac{1}{2}$  hours. The average time for the six radish roots was  $1\frac{1}{3}$  hours with a greater range of variation, being from  $\frac{3}{4}$  of an hour to  $2\frac{1}{2}$  hours.

3. The rate of elongation of root hairs on any one root is quite uniform.

From observations made during these investigations it would appear that the slight differences in length of root hairs in any one region of the root are due more to the age of the root hairs, that is, to the relative time of initiation, than to their rate of growth. The different lengths of root hairs found at about the same level, shown in the photograph of a radish root (fig. 4, Pl. LXII), are due to differences in age rather than to differences in rate of elongation. Sometimes one or more root hairs will develop for some time before others start in their vicinity. This gives an irregularity in length which might be thought due to a difference in the growth rate.

4. Of the two species studied, the root hairs grew more slowly on the one in which they were produced in greatest abundance.

A much larger proportion (three or four times as many) of the epidermal cells of the corn produced root hairs than did those of the radish (compare figs. 5 and 6, Pl. LXII). After lateral movement ceased, the radish root hairs studied grew at a rate varying for the most part from 31 to 50 microns per 15-minute interval, with an average rate of 41 microns. The rate of root-hair growth in the corn under the same conditions varied from 29 to 38 microns per 15-minute interval, with an average rate of 35 microns. This shows that the radish root hairs, which comparatively speaking are scattered, grow more rapidly than do those of the corn.

#### THE EFFECT OF LIGHT ON THE RATE OF ROOT-HAIR ELONGATION

The effect of light on the direction and rate of growth of plant parts has been studied by Sachs (38), Duchartre (14), Hofmeister (19), Pfeffer (35), and others. The facts that internodes of stems often grow more rapidly in the dark than when exposed to light, and that shoots tend to bend toward the source of a one-sided illumination, have been taken as proof that light has a retarding effect upon growth. Exceptions to this are found, however, as in the case of leaves, tendrils, and certain internodes, which elongate more on the side exposed to light.

It is within the last fifteen years that the most careful work on the effect of light on growth has been carried on. Much of this has been a study of phototropic bending in multicellular organs. The coleoptiles of cereals, particularly of *Avena sativa*, have been used extensively in these investigations because of their great sensitivity to the light stimulus. Clark (10), Arisz (1), Vogt (48), Sierp (42), Brauner (9), and others have shown a positive bending of this structure under the influence of light, indicating that light retards growth.

Other plant parts used have been the hypocotyls and roots of various seedlings. Blaauw (6) worked with the hypocotyl of the sunflower (*Helianthus globosus*) and obtained, on the whole, a retardation of growth in the light. His results with the roots of *Lepidium sativum*, *Avena sativa*, *Raphanus sativus*, and *Sinapis alba* were not so consistent. Lighting with 130,000

M. C. S., which produced a strong reaction of the *Helianthus hypocotyl*, led to a scarcely measurable retardation in the *Lepidium* root. With *Avena sativa* he got even less evidence of a retardation. This seemed to him rather surprising inasmuch as the coleoptile of this plant is so extremely sensitive to light. He then tested *Raphanus sativus* and says that the root of this plant is so insensitive to light that even with a lighting of 2000 M. C. during four hours it showed no light-growth reaction and continued to grow as in the dark. Lastly he used the root of *Sinapis alba* and obtained a light-growth reaction, quite strongly developed in 1500 M. C. and clearly evident in 64 M. C., but weak and questionable in 8 M. C. The decrease in growth rate in the *Sinapis alba* root in 1500 M. C. was about the same as in the *Helianthus hypocotyl* in one M. C. Blaauw then estimates the sensitivity of the *Helianthus hypocotyl* to be at least one thousand times that of the root of white mustard.

Such studies as these, while very important in the study of the effect of light upon complex tissue systems, contribute only slightly to the question of the effect of light on cell processes. Variation in the growth rate must be primarily produced by cellular changes. A decrease in the rate of elongation of a multicellular tissue may be the result of a longer time required for the cells to enlarge, or it may be due to a longer time required for cell-division to take place. The following investigations have been carried on in order to determine if the effect of light in retarding the rate of growth in the higher plants is an effect on cell-enlargement or on cell-division.

Previous work of this nature has been confined to the lower plants, particularly the *Mucorales*. Lendner (28) noted that the sporophores of many members of this group become elongated in the dark, light having a retarding effect on their development. Probably the most important work of this kind is that of Blaauw (5) on the sporophores of *Phycomyces nitens*. Blaauw got on the whole an acceleration in growth in 1- and 64-meter candle power, while in higher intensities (4000 M. C.) a retardation was noted. It is to be remembered that these *Mucorales* are all coenocytic and thus differ from the uninucleate root hairs. It may even be that the effect of light in retarding enlargement of these hyphae is an effect on nuclear division rather than on the process of cell-enlargement *per se*.

The work already done on light in its relation to root hairs has been confined to a study of its effect on their abundance. Schwarz (40) found that light had no effect on hair-production in *Zea mays* and *Pisum sativum*. Devaux (12) found that light retarded elongation of the cells of the root of *Zea mays* and favored hair-production. Miss Snow (44), working with *Triticum vulgare*, *Pisum sativum*, and *Cucurbita Pepo*, reached the conclusion that light or darkness has little effect on root-hair production, and any slight effect found she considered due to an indirect effect upon growth.

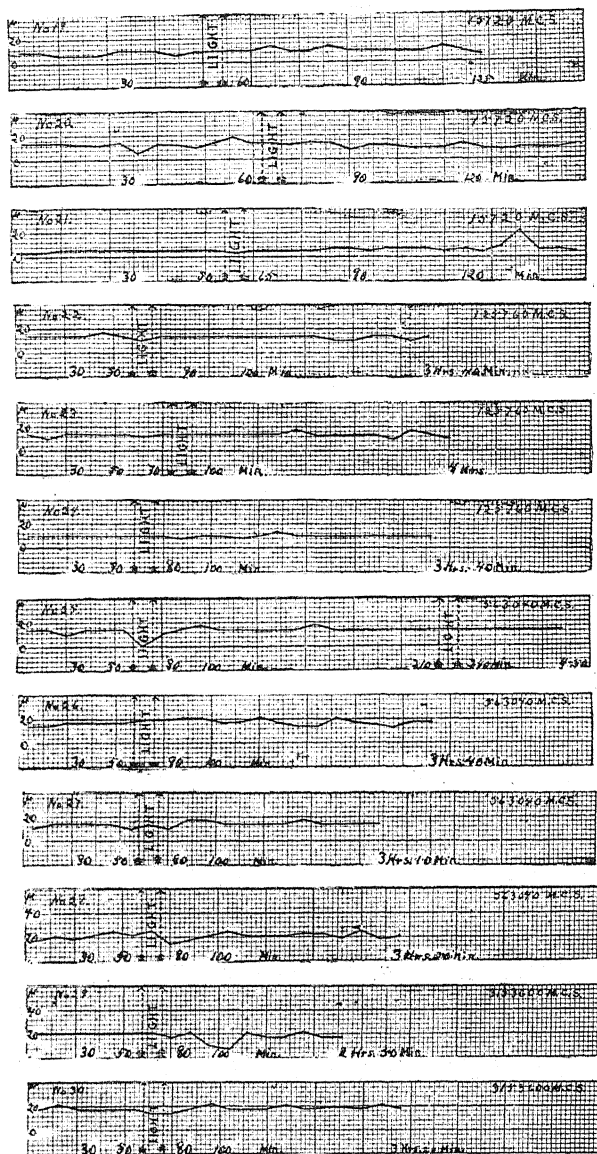
Root-hairs of *Raphanus sativus* L. and *Sinapis alba* L. were used in this study. These were grown as described above for the investigations on the

grand period of growth, the work being carried on in a constant-temperature room. The light was obtained from (Mazda) gas-filled frosted bulbs, with the exception of the strongest illumination which was obtained from a large clear bulb made for use in projecting lanterns. The lamps were carefully calibrated, so their exact candle power was known. The bulb was placed in a projecting lantern and the horizontal microscope placed before it at a given distance, so that the light, when turned on, fell directly upon the root mounted on the stage. A thermometer was placed beside the mount in order that any change in temperature might be noted.

In each case a root hair was chosen, the lateral movement of which had ceased, and which therefore was practically constant in its growth rate in the dark. The rate of growth was recorded for an hour before the light was applied, in order to be sure that growth in the dark was uniform. Readings were taken at from 5- to 10-minute intervals, the light being turned on during one period except when continuous lighting was used. The distance between light and object was carefully measured.

Lists numbered 19-30 below, and graphs 19-30 (text fig. 3) present the results obtained from a study of 12 root hairs of radish (*Raphanus sativus* L.).

- No. 19. Interval 5 min. Temp. 15.5° C. Illumination 15720 M. C. S.: 10.5; 10.5; 7.0; 7.0; 10.5; 10.5; 10.5; 7.0; 8.7; (Light 10.5); 10.5; 10.5; 14.0; 10.5; 10.5; 14.0; 10.5; 10.5; 10.5; 10.5; 10.5; 14.0; 10.5; 7.0.
- No. 20. Interval 5 min. Temp. 16° C. Illumination 15720 M. C. S.: 14.0; 14.0; 14.0; 12.3; 12.3; 14.0; 5.3; 12.3; 12.3; 10.5; 14.0; 19.3; 14.0; (Light 14.0); 12.3; 14.0; 14.0; 8.8; 12.3; 12.3; 10.5; 10.5; 10.5; 14.0; 10.5; 8.8; 10.5; 10.5; 10.5; 12.3.
- No. 21. Interval 5 min. Temp. 16°-16.5° C. Illumination 15720 M. C. S.: 3.5; 3.5; 5.3; 5.3; 5.3; 5.3; 5.3; 5.3; 5.3; 5.3; 5.3; 5.3; (Light 3.5); 5.3; 5.3; 5.3; 5.3; 7.0; 7.0; 5.3; 7.0; 7.0; 5.3; 7.0; 7.0; 5.3; 7.0; 5.3; 8.8; 22.8; 7.0; 7.0; 5.3; 7.0; 7.0; 5.3.
- No. 22. Interval 10 min. Temp. 17.5° C. Illumination 125760 M. C. S.: 14.0; 14.0; 14.0; 14.0; 17.5; 14.0; (Light 10.5); 14.0; 14.0; 14.0; 14.0; 14.0; 14.0; 14.0; 14.0; 14.0; 10.5; 10.5; 14.0; 14.0; 10.5; 14.0.
- No. 23. Interval 10 min. Temp. 13.5° C. Illumination 125760 M. C. S.: 14.0; 10.5; 14.0; 14.0; 14.0; 14.0; 12.3; 14.0; (Light 14.0); 14.0; 14.0; 14.0; 14.0; 14.0; 17.5; 14.0; 14.0; 14.0; 14.0; 10.5; 14.0; 17.5; 14.0; 10.5.
- No. 24. Interval 10 min. Temp. 14° C. Illumination 125760 M. C. S.: 10.5; 10.5; 10.5; 10.5; 10.5; (Light 10.5); 10.5; 8.8; 10.5; 10.5; 8.8; 10.5; 14.0; 10.5; 10.5; 10.5; 10.5; 10.5; 10.5; 10.5.
- No. 25. Interval 10 min. Temp. 14.5° C. Illumination 563040 M. C. S.: 14.0; 14.0; 10.5; 14.0; 14.0; 14.0; (Light 00.0); 10.5; 14.5; 17.5; 14.0; 14.0; 14.0; 14.0; 14.0; 17.5; 14.0; 14.0; 14.0; 14.0; 14.0; 14.0; (Light 14.0); 14.0; 14.0; 14.0; 14.0; 14.0; 14.0.
- No. 26. Interval 10 min. Temp. 16° C. Illumination 563040 M. C. S.: 14.0; 14.0; 17.5; 17.5; 17.5; 17.5; (Light 19.3); 19.3; 21.0; 21.0; 17.5; 17.5; 21.0; 17.5; 14.0; 14.0; 21.0; 17.5; 15.8; 12.3; 17.5; 17.5.
- No. 27. Interval 10 min. Temp. 16° C. Illumination 563040 M. C. S.: 10.5; 14.0; 14.0; 14.0; 10.5; (Light 14.0); 10.5; 17.5; 17.5; 14.0; 14.0; 14.0; 14.0; 17.5; 14.0; 14.0; 14.0; 14.0.
- No. 28. Interval 10 min. Temp. 16°-17.5° C. Illumination 563040 M. C. S.: 17.5; 21.0; 17.5; 21.0; 24.5; 21.0; (Light 24.5); 14.0; 17.5; 21.0; 24.5; 21.0; 21.0; 21.0; 22.8; 22.8; 19.3; 24.5; 17.5; 21.0.



TEXT FIG. 3. Graphs showing the result of the application of light to twelve root hairs of radish, illumination varying from 15720 M. C. S. to 3153600 M. C. S. For discussion see text.

- No. 29. Interval 10 min. Temp. 19.5°-22° C. Illumination 3153600 M. C. S.: 21.0; 21.0; 21.0; 21.0; 21.0; (Light 21.0); 17.5; 21.0; 10.5; 7.0; 21.0; 17.5; 17.5; 21.0; 17.5; 17.5.
- No. 30. Interval 10 min. Temp. 16°-19° C. Illumination 3153600 M. C. S.: 17.5; 21.0; 17.5; 17.5; 17.5; 17.5; (Light 14.0); 14.0; 17.5; 21.0; 17.5; 17.5; 17.5; 21.0; 17.5; 19.3; 19.3; 17.5; 21.0; 17.5.

An examination of no. 19, with a lighting of 15720 M. C. S., shows no retardation but instead a slight acceleration at three points. This acceleration is noted during one 5-minute interval, the rate returning to normal during the following 5 minutes. These variations are no greater than those noted in root hairs grown in the dark (nos. 1-14).

No. 20 presents the growth of a root hair showing more variation in the growth rate than does no. 19. Two minima occurred after exposure, but these can not be considered a light effect, for a retardation of even greater amount is noted before the root was illuminated.

The root hair represented by no. 21 has a quite uniform growth except for a 10-minute interval about  $1\frac{1}{4}$  hours after light was applied. During this time growth was greatly accelerated for 5 minutes, returning to normal during the next 5 minutes. Because of the time which has elapsed since lighting, and because of the fact that no corresponding acceleration occurs in the other root hairs growing under the same illumination, this variation can not be considered as being due to light. Similar sudden variations in the growth rate, lasting for from 10 to 15 minutes, have been noted by the writer in root hairs growing in continuous darkness.

Nos. 22, 23, and 24, illumination 125760 M. C. S., show great uniformity in growth rate both before and after lighting. No. 22 shows a slight retardation during the lighting period and another slight retardation for about 20 minutes, over  $1\frac{1}{2}$  hours later. No. 24 shows two 10-minute periods of slight retardation, one 10 minutes after lighting and another 20 minutes later. On the whole, the slight variations are no more than might be expected were light not applied.

No. 25, illumination 563040 M. C. S., shows a complete cessation of growth in length after light is applied. However, this growth is resumed in the dark and returns to the normal rate inside of 20 minutes. During this interval of suspended elongation the root hair swelled at the tip, so that growth continued as an increase in diameter rather than in length. That this stoppage in elongation is due to the effect of light is questionable. The swelling at the tip would indicate a temperature effect, as shown in later studies. However, no rise in temperature was noted here. It is seen that the light when applied  $2\frac{1}{2}$  hours later to the same root hair for the same period of time produced no variation in its growth rate.

No. 26, with the same illumination as no. 25, shows an acceleration of growth following exposure to light. This is followed nearly  $1\frac{1}{2}$  hours later by a retardation amounting to 7 microns per interval, within 20 minutes. This retardation lasts through two 10-minute intervals. However, the rate here is no slower than that observed during the first two periods in darkness, so that it can not be considered of any significance.

No. 28 shows some irregularities in growth both before and after lighting. The rate in darkness varies from 17 to 24 microns per 10-minute interval, and the same variations are found after lighting except in the period immediately following, when the growth is but 14 microns.



In the studies under strong lighting the temperature factor was not entirely eliminated. A thermometer standing just in front of the moist chamber showed an increase in temperature of from  $2\frac{1}{2}$  to 3 degrees C., during the 10-minute interval that light was applied. In most cases a swelling of the tip occurred (fig. 3, Pl. LXII). During this interval of swelling, growth in length was greatly retarded. It is entirely likely that this swelling was due not to the effect of strong light but to increase in temperature, which will be shown later to have a marked effect on root-hair growth.

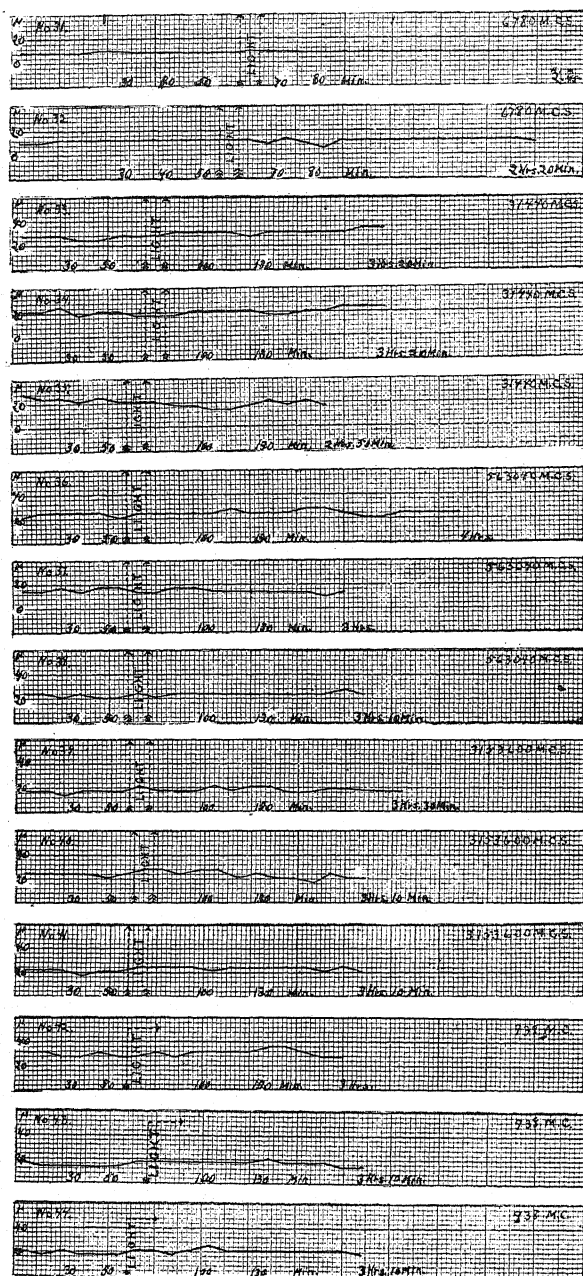
In no. 29, illumination 3153600 M. C. S., we find a decided retardation, with swelling of the tip, coming about 40 minutes after lighting. It is questionable whether this is the result of lighting, change of temperature, or some other unknown cause. Such peculiarities in the growth rate do occasionally occur in root hairs grown in the dark under uniform conditions of temperature and moisture.

No. 30, illumination as before, shows a slight retardation during the application of light and during the 10-minute interval following. This retardation was accompanied by some swelling of the tip. Following this, growth was much the same as before illumination.

It appears then that *the root hairs of Raphanus sativus, under light intensities ranging from 15720 M. C. S. to 563040 M. C. S., show no light-growth reaction.* It is also likely that much brighter illumination (3153600 M. C. S.) has no effect. Variations which do occur after lighting come at no uniform intervals and are no greater than when the root hairs are grown continuously in the dark.

Finding apparently no reaction to light in the root hair of *Raphanus sativus*, *Sinapis alba* was next investigated. The root of this plant, being the one in which Blaauw (7) obtained positive evidences of retardation in growth, would seemingly be the most satisfactory of any known for this purpose. Here four light intensities of short duration and one series under continuous lighting were used. Lists numbered 31-44 and graphs 31-44 (text fig. 4) show the results of these experiments.

- No. 31. Interval 5 min. Temp.  $16^{\circ}$  C. Illumination 6780 M. C. S.: 8.8; 8.8; 8.8; 8.8; 10.5; 10.5; 8.8; 8.8; 8.8; 8.8; 8.8; 8.8; (Light 10.5); 9.7; 9.7; 8.8; 8.8; 8.8; 8.8; 8.8; 8.8; 8.8; 8.8; 8.8; 8.8.
- No. 32. Interval 5 min. Temp.  $20^{\circ}$  C. Illumination 6780 M. C. S.: 10.5; 10.5; 12.3; 12.3; 12.3; 12.3; 12.3; 12.3; 12.3; 12.3; (Light 12.3); 12.3; 9.8; 14.0; 10.5; 5.3; 12.3; 12.3; 12.3; 12.3; 12.3; 12.3; 12.3; 12.3; 12.3; 12.3; 10.5.
- No. 33. Interval 10 min. Temp.  $18^{\circ}$  C. Illumination 31440 M. C. S.: 28.0; 28.0; 28.0; 24.5; 24.5; 28.0; 28.0; (Light 28.0); 31.5; 31.5; 31.5; 31.5; 28.0; 31.5; 31.5; 31.5; 31.5; 31.5; 35.0; 35.0.
- No. 34. Interval 10 min. Temp.  $18^{\circ}$ - $18.5^{\circ}$  C. Illumination 31440 M. C. S.: 21.0; 21.0; 24.5; 17.5; 21.0; 21.0; 17.5; (Light 17.5); 17.5; 17.5; 21.0; 21.0; 21.0; 21.0; 17.5; 17.5; 21.0; 21.0; 24.5; 24.5.
- No. 35. Interval 10 min. Temp.  $16^{\circ}$  C. Illumination 31440 M. C. S.: 28.0; 24.5; 24.5; 21.0; 24.5; 21.0; (Light 21.0); 21.0; 17.5; 17.5; 14.0; 14.0; 17.5; 21.0; 17.5; 21.0; 17.5.



TEXT FIG. 4. Graphs 31-41 show the result of the application of light to eleven root hairs of white mustard, illumination varying from 6780 M. C. S. to 315360 M. C. S. Graphs 42-44 show the result of continuous application of light (938 M. C.) over a period of 2 hours to three root hairs of white mustard. For discussion see text.

- No. 36. Interval 10 min. Temp.  $16^{\circ}$ – $17^{\circ}$  C. Illumination 563040 M. C. S.: 21.0; 24.5; 24.5; 24.5; 21.0; (Light 24.5); 24.5; 24.5; 24.5; 24.5; 28.0; 24.5; 24.5; 24.5; 28.0; 28.0; 24.5; 21.0; 21.0; 24.5; 24.5; 24.5.
- No. 37. Interval 10 min. Temp.  $16^{\circ}$ – $16.5^{\circ}$  C. Illumination 563040 M. C. S.: 14.0; 14.0; 17.5; 14.0; 17.5; 17.5; (Light 14.0); 14.0; 17.5; 17.5; 14.0; 14.0; 14.0; 14.0; 14.0; 10.5; 14.0.
- No. 38. Interval 10 min. Temp.  $17^{\circ}$  C. Illumination 563040 M. C. S.: 24.5; 24.5; 21.0; 24.5; 21.0; 21.0; (Light 24.5); 21.0; 24.5; 24.5; 24.5; 24.5; 24.5; 24.5; 24.5; 24.5; 24.5; 28.0; 24.5.
- No. 39. Interval 10 min. Temp.  $16^{\circ}$ – $19^{\circ}$  C. Illumination 3153600 M. C. S.: 17.5; 17.5; 14.0; 17.5; 17.5; 17.5; (Light 21.0); 17.5; 17.5; 17.5; 21.0; 17.5; 21.0; 21.0; 17.5; 17.5; 19.3; 19.3; 17.5; 17.5; 17.5.
- No. 40. Interval 10 min. Temp.  $18.5^{\circ}$ – $21^{\circ}$  C. Illumination 3153600 M. C. S.: 24.5; 24.5; 24.5; 21.0; 24.5; (Light 28.0); 28.0; 24.5; 28.0; 28.0; 21.0; 24.5; 21.0; 21.0; 17.5; 24.5; 21.0; 21.0.
- No. 41. Interval 10 min. Temp.  $18^{\circ}$  C. Illumination 3153600 M. C. S.: 21.0; 21.0; 21.0; 17.5; 21.0; 21.0; (Light 24.5); 24.5; 24.5; 24.5; 21.0; 24.5; 24.5; 24.5; 24.5; 24.5; 21.0; 24.5; 21.0.
- No. 42. Interval 10 min. Temp.  $18^{\circ}$ – $18.5^{\circ}$  C. Illumination 938 M. C.: 31.5; 31.5; 28.0; 28.0; 31.5; 28.0; (Light 28.0; 31.5; 28.0; 31.5; 31.5; 31.5; 31.5; 35.0; 35.0; 31.5; 28.0; 28.0).
- No. 43. Interval 10 min. Temp.  $15.5^{\circ}$ – $17.5^{\circ}$  C. Illumination 938 M. C.: 17.5; 14.0; 14.0; 14.0; 14.0; 17.5; (Light 17.5; 17.5; 17.5; 17.5; 17.5; 17.5; 21.0; 17.5; 17.5; 17.5; 14.0; 14.0).
- No. 44. Interval 10 min. Temp.  $15^{\circ}$ – $16.5^{\circ}$  C. Illumination 938 M. C.: 21.0; 17.5; 21.0; 17.5; 17.5; 17.5; (Light 21.0; 21.0; 17.5; 21.0; 24.5; 21.0; 21.0; 21.0; 21.0; 21.0; 21.0; 21.0; 17.5).

No. 31, illumination 6780 M. C. S., shows great uniformity of growth in light. No. 32 shows two decided intervals of retardation following light. That this is due to light may be questioned, as no such retardation is shown in the previous study or in those following.

No. 33, illumination 31440 M. C. S., shows little variation in growth after lighting. In general, the rate of growth is more rapid after the lighting period. No. 34 shows variations equaling 7 microns per period after light. Equal variations may be noted in the hour just preceding lighting, while the root hair was still in darkness. No. 35 shows a retardation to 14 microns per interval about 30 minutes after lighting. This is 7 microns per interval less than recorded in the dark. If it were not for the two preceding investigations which show no such retardation, it might be suspected of being a light-growth reaction.

No. 36, illumination 563040 M. C. S., shows much the same variations after lighting as while in darkness. There are two periods of more rapid growth following the application of light. Both nos. 37 and 38 show great uniformity of growth.

With an illumination of 3153600 M. C. S., trouble was encountered with increased temperature as in the case of *Raphanus sativus* under the same intensity. Swelling was very common, the root hairs in this case ceasing to elongate within a few minutes, making them useless for study. Con-

sequently three root hairs were chosen which did not swell at the tip during the application of light. Their rates of growth are given in lists nos. 39, 40, and 41. With the exception of no. 40, none show more retardation after lighting than in continuous darkness. The slight retardation in no. 40 appears  $1\frac{1}{2}$  hours after lighting and lasts but one interval.

Obtaining no positive results from short periods of lighting, continuous lighting was finally tried. An intensity of 938 M. C. was used. A light intensity greater than this could not be applied under the conditions, without increasing the temperature to an extent which would interfere with the experiment. By examining nos. 42, 43, and 44, it can be seen that in no case did the rate of growth after exposure drop below the growth rate in the dark. The oscillations following the application of light resemble closely the variations while grown in the dark.

In conclusion it may be said that *neither the root hairs of Raphanus sativus nor those of Sinapis alba show any reaction definitely attributable to light up to an intensity above 3153600 M. C. S., and Sinapis alba shows no such reaction in continuous lighting for two hours under an intensity of 938 M. C.*

It may be asked why the root hairs of *Sinapis alba* fail to show any light-growth reaction, while the root, as found by Blaauw (7), shows a decided retardation under light. An answer may be found by determining whether light influences cell-division or cell-elongation. Sachs (38) investigated certain algae (*Spirogyra*, *Ulothrix*, *Hydrodictyon*, and others) and concluded that cell-division takes place chiefly at night in these forms. He also states that in *Pilobolus crystallinus* the splitting of the protoplasm in the sporangium into spores takes place only at night, the spores being thrown out in the light. He concludes from these studies that light retards cell-division. Later investigations of this problem have not been convincing. Karsten (21, 22) studied carefully the rate of cell-division in light and darkness in roots, stems, and leaves, and also in algae. He examined his material at intervals to determine the proportion of dividing to resting cells. His results were not very conclusive. Friesner (15), probably the most recent careful investigator of this question of periodicity of cell-division, concludes that the times of maximum and minimum cell-division in a root are dependent upon the time of initiation of the metabolic processes, not upon alternating periods of light and darkness. Berinsohn (4), working with the roots of *Allium Cepa*, determined by sectioning the proportion of dividing to resting cells in roots grown in the light. He compared these results with those obtained from roots grown in the dark. He found a larger proportion of dividing cells in roots grown in the dark, and concluded that light has a retarding effect on cell-division. If light has such a retarding effect, we should have this factor entering and tending to decrease the rate of growth.

It may be pointed out that in the root there is a complex tissue composed of thousands of cells. Light may have a retarding effect on the elongation of

these cells which, multiplied many times as it would be in the root, could be easily measured. However, this retardation as expressed in an individual cell (as the root hair) may be too slight to be measured by the methods employed in this investigation, or it may be covered by fluctuating variations in the growth rate. The fact, however, that many of the root hairs investigated exhibited rather an increase than a decrease in their growth rate after light was applied, would lead one to question the retarding effect of light on cell-elongation.

There are several possible sources of error in an investigation such as this. It is impossible to read with absolute certainty the amount of root-hair elongation by the use of an eyepiece micrometer, because (1) the root hair may not be growing exactly at right angles to the line of vision; (2) the small size of the micrometer scale and root hair makes it impossible to be absolutely certain of the exact location of the root-hair tip; (3) it is impossible to read fractions of spaces on the micrometer scale with any degree of accuracy. It may be that, with the development of more delicate apparatus for measuring the growth of the cell, and with stronger light intensities than were used in the investigations here reported, a light-growth reaction may be discovered in root-hair cells. But from the evidence thus far obtained it must be held, for the present at least, that light has no effect upon the enlargement of root-hair cells.

#### THE EFFECT OF TEMPERATURE ON THE RATE OF ROOT-HAIR ELONGATION

The first work of importance on the relation of temperature to the growth processes in higher plants is that of Sachs (37), who about 1860 studied the effect of temperature on germination. He measured the amount of growth of soaked seeds for a given period at a given temperature. Prior to the experiment the seeds were soaked usually for about 48 hours, the time being measured from the end of the soaking. Miss Leitch (27) points out the defects in this method, an important one being that there is great variation in the rate at which roots of the same species, and treated in the same manner, burst through their seed coats.

Koeppen (23) was the next important student of this problem. Working with a number of different seedlings, including maize, he obtained varying results. His curve for maize as well as those for many other plants shows two optima. Lehenbauer (26), one of the more recent investigators, using more refined methods found no indication of the double optimum. Other investigators, as Sachs (37) and Davenport (11), have described the curve for maize as having a single optimum. Lehenbauer from his work on maize concludes that the term "optimum temperature for growth" means little unless the time of exposure is definitely stated. He found with high temperatures (31° C. and above) a decrease in the growth rate with prolonged periods of exposure, while this did not occur with temperatures near the minimum (12°–14° C.). Miss Leitch (27) considers that the term

"optimum" should be used for the highest temperature at which growth takes place at a constant rate. She introduces the term "maximum-rate temperature" for the temperature at which growth reaches its highest intensity over a short period of time.

As regards the influence of a sudden change of temperature on growth, Askenasy (2) found this dependent on the position of the lower limit used. If the lower limit still permitted growth, a change to a higher temperature resulted in immediate increase in the growth rate. When, however, the minimum temperature used was near the zero point of growth for the plant studied, an increase in temperature was followed by a more or less tardy resumption of the normal growth rate. Pfeffer (35) noted similar results in some of his investigations. Godlewski (16) found in the case of the epicotyl of *Phaseolus vulgaris* that an exposure to sudden changes of temperature, either upward or downward, resulted in a temporary decrease in the growth rate. True (45), working with *Vicia faba*, found that a transfer of the radicle to a suddenly lowered temperature (near 0° C.) resulted not only in a checking of elongation but in actual contraction. Measurements at short intervals showed that this contraction lasted for five minutes. The root remained at a standstill for about one half hour, then began to elongate slowly and irregularly. Being brought back to normal temperature, the root returned to about two thirds its normal growth rate. Between the temperatures of 18° and 30° C. he did not consider that a change in temperature produced any very noticeable change in growth. There was some checking in the growth rate during the first 15 minutes, followed by an increased rate of elongation during the second 15 minutes. He considers this retardation a turgor change. Pfeffer (35) has found experimentally that the turgor pressure is influenced by temperature changes in the same manner as gas pressure.

The work on temperature in its relation to root hairs, as in the case of light, has been confined to studies of its effect on the presence or absence of hairs and on their abundance if present. Miss Snow found (44) that with high temperatures and sufficient moisture there was a tendency to decrease the number of root hairs produced in consequence of the increased ventral elongation of the cells of the root. Wollny (49) reported an increase in the number of hairs on aerial organs due to aerial transpiration. Here transpiration may affect hair-production through its influence on plant temperatures.

The purpose of the investigations described below was to determine if slight changes in temperature, between the limits of 17° and 27° C., would affect the rate of elongation of a root hair which had been growing at a constant temperature. These temperatures, while somewhat below the optimum given for most plants, still approach it and are removed some distance from the minimum.

As in the study of the effect of light on growth, roots of *Raphanus sativus*

and *Sinapis alba* were used. These were grown in the same manner as previously described, the investigations being carried on in the dark in the same constant-temperature room. A temperature chamber was devised which could be attached to the stage of the microscope (fig. 2, Pl. LXII), and the moist chamber containing the growing roots was placed in this. The thermometer was passed through an opening in the top of the temperature chamber, its bulb being inserted in a small vial surrounded by a layer of saturated filter paper. This imitated rather closely the existing conditions in the moist chamber. A small opening in the side of the temperature chamber, of the proper size to admit the objective of the microscope, enabled the observer to measure the growth rate of the root hairs by means of the eyepiece micrometer. The rate of growth was observed at 5- to 10-minute intervals for a half hour or more. For a cooling solution a bottle containing crushed ice was placed in the temperature chamber, care being taken not to disturb the mounted root hair. For a heating solution a bottle was partly filled with water and just before inserting it in the chamber 10 or more cc. of concentrated sulfuric acid were poured into this. The reaction produced developed heat sufficient to raise the temperature of the chamber  $2\frac{1}{2}$  to 3 degrees C. and rarely even more, within 10 to 15 minutes.

Lists numbered 45-47 show the effect of the cooling solution on root-hair elongation in *Sinapis alba*. Readings were taken at 10-minute intervals in nos. 45 and 46, and at 5-minute intervals in no. 47. *Below each successive increment is given the temperature in italics.*

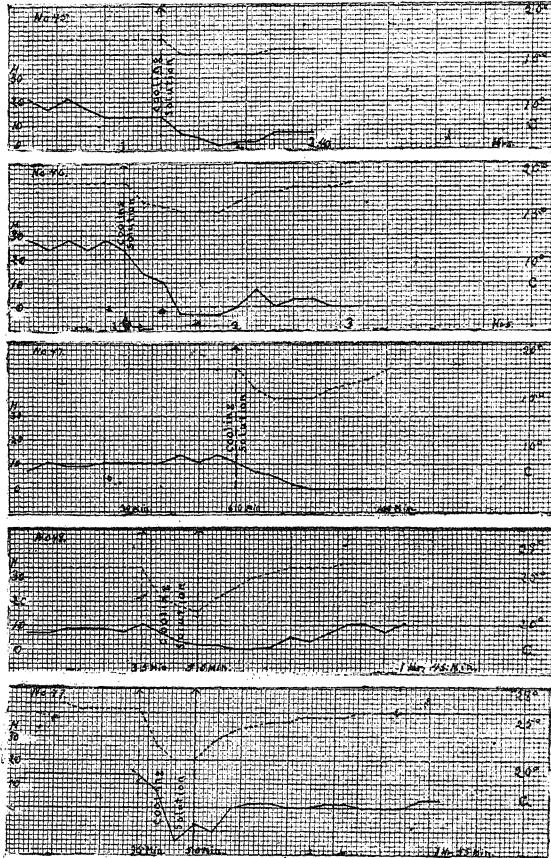
- No. 45. 21.0; 17.5; 21.0; 17.5; 14.0; 14.0; 14.0; 14.0; (Cooling solution 7.0; 5.3; 1.8; 3.5;  
17 17 17 17 17 17 17 ( 15.5 15.5 15.5 15.5  
3.5; 7.0; 7.0; 7.0).  
15.5 16 16 16)
- No. 46. 28.0; 24.0; 28.0; 24.0; 28.0; 24.0; (Cooling solution 14.0; 10.5; -3.5; -3.5; -3.5;  
18.5 18.5 18.5 18.5 18.5 18.5 ( 16.5 16 15.5 15.5 15.5  
0.0; 7.0; 0.0; 3.5; 3.5; 0.0; 0.0).  
16.5 17.5 17.5 18 18 18 18.5)
- No. 47. 7.0; 10.5; 8.8; 8.8; 10.5; 10.5; 10.5; 10.5; 14.0; 10.5; 14.0; 10.5; (Cooling solution  
18 18 18 18 18 18 18 18 18 18 18 ( 7.0; 5.3; 1.8; 0.0; 0.0; 0.0; 0.0; 0.0).  
16 15 15 15 16 16.5 17 18)

Lists numbered 48-51 show the effect of the cooling solution on root-hair elongation in *Raphanus sativus*. The interval in each case is 5 minutes.

- No. 48. 7.0; 7.0; 8.8; 8.8; 8.8; 7.0; 10.5; (Cooling solution 7.0; 3.5; 1.8); 1.8; 0.0; 0.0; 1.8;  
26 26 26 26 26 26 26 ( 23 22 21.5) 23 24 25 25.5  
5.6; 3.5; 7.0; 10.5; 10.5; 7.0; 10.5.  
26 26 26 26.5 26.5 26.5 26.5
- No. 49. 14.0; 14.0; 14.0; 14.0; 14.0; 14.0; 14.0; 14.0; (Cooling solution 7.0; 14.0; -7.0); -10.0;  
27 27 27 26.5 26.5 26.5 26.5 ( 22.5 21 21 ) 23  
0.0; 1.8; 1.8; 0.0; 0.0; 1.8; 1.8; 0.0; 0.0; 0.0; 3.5; 3.5.  
24 24.5 25 25 25.5 25.5 25.5 26 26 26 26 26
- No. 50. 14.0; 17.5; 14.0; 17.5; 17.5; 17.5; 14.0; (Cooling solution 7.0; 0.0; 0.0).  
27 26.5 26.5 26.5 26.5 26 26 ( 25 24 24)

No. 51. 14.0; 14.0; 14.0; 17.5; 17.5; 17.5; 14.0; (Cooling solution 10.5; 10.5; 3.5; 0.0).  
 27 27 26.5 26.5 26.5 26.5 26.5 ( 25 25 25 25 )

No. 52 shows the effect of the heating solution on a root hair of *Sinapis alba*; nos. 53 and 54 show the effect of a similar solution on root-hair elongation in *Raphanus sativus*. Interval 5 minutes in each.



TEXT FIG. 5. Numbers 45-47 show the effect of a cooling solution on the rate of root-hair elongation in white mustard. Nos. 48 and 49 show the effect of a similar solution on the elongation of root hairs of radish. The solid line gives the rate of root-hair elongation, the broken line the temperature.

No. 52. 21.0; 21.0; 24.5; 24.5; 21.0; 21.0; 21.0; 24.5; 24.5; 24.5; (Heating solution 17.5;  
 25 25 25 25 25 25 25 25 25 25 ( 27  
 38.5; 17.5; 7.0; 0.0; 0.0).  
 30 32.5 32 32 32 )

No. 53. 10.5; 14.0; 10.5; 14.0; 14.0; 10.5; 10.5; (Heating solution 7.0; 17.5; 14.0; 14.0;  
 25 25 25 25 25 25 25 ( 26 26.5 26.5 27  
 10.5; 17.5; 17.5).  
 27.5 27.5 28 )



No. 54. 14.0; 17.5; 17.5; 17.5; 17.5; 14.0; (Heating solution 21.0; 28.0; 21.0); 14.0; 14.0;  
 26 26 26.5 26 26 26 ( 27.5 28.5 29 ) 28.5 27.5  
 14.0; 14.0; 17.5; 14.0; 14.0; (Heating solution 21.0; 24.5; 17.5); 10.5; 10.5; 14.0; 14.0;  
 27 27 27 26.5 26.5 ( 27.5 28.5 29 ) 28 27.5 27 27  
 14.0; 17.5; 17.5; 17.5; 17.5.  
 26.5 26.5 26.5 26.5 26.5

No. 45 shows during the first 10 minutes after a drop of  $1.5^{\circ}$  C. a retardation of elongation to one half of its former rate, that is, from 14 to 7 microns. After 20 minutes more, the temperature remaining constant, the rate of growth dropped to 1.8 microns per interval. It increased during the next two intervals to 3.5 microns, and at the end of one hour was again 7 microns per interval, which rate was maintained for at least 20 minutes longer.

No. 46, with a cooling of two degrees ( $18.5^{\circ}$ – $16.5^{\circ}$  C.), shows a reduction in the growth rate of 10 microns (24 to 14 microns) during the first 10 minutes. At the end of 30 minutes, with a temperature of  $15.5^{\circ}$  C., a shortening of the root hair was noted. This amounted to 3.5 microns for the 10-minute interval, and was continued through three intervals at the same rate. Movement then ceased, and later, with an increase in temperature, some elongation was again noted. However, this was little more than sufficient to bring the root hair back to its length before contraction began.

No. 47 shows a reduction in the growth rate from 10.5 microns to 7 microns per interval during the first 5 minutes after a cooling of two degrees ( $18^{\circ}$  to  $16^{\circ}$  C.). The rate then decreased gradually with a drop of one degree, and at the end of 20 minutes, at  $15^{\circ}$  C., growth had ceased and was not resumed during the period of observation. No contraction was noted.

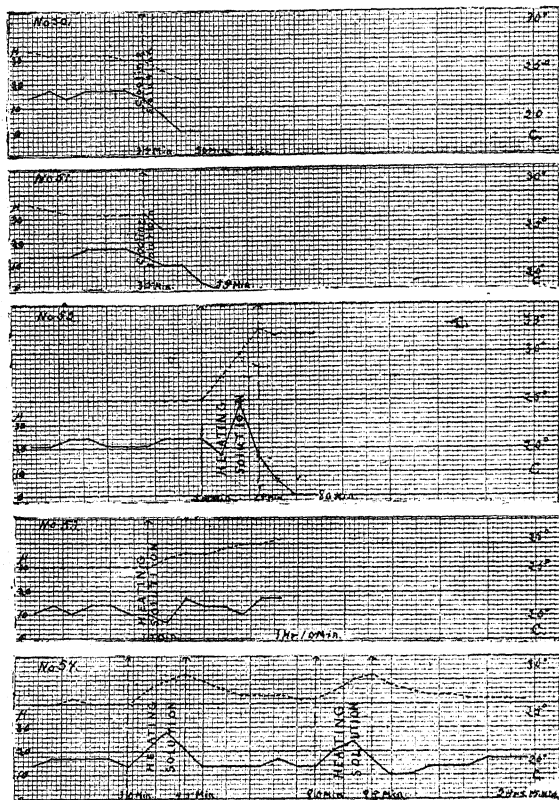
No. 48 shows the results of applying the cooling solution during 15 minutes. In this time the temperature was gradually reduced from  $26^{\circ}$  to  $21.5^{\circ}$  C. At the same time we find a gradual reduction in the growth rate from 10.5 microns per interval just before cooling to 1.8 microns at the time the cooling solution was removed. Although the temperature rose during the next 10 minutes  $2\frac{1}{2}$  degrees (from  $21.5^{\circ}$  to  $24^{\circ}$  C.), the rate of growth continued to decrease, and finally growth ceased for a period of 10 minutes. It was then slowly resumed at a temperature of  $25.5^{\circ}$ , and 15 minutes later had returned to normal.

No. 49 shows a decided contraction of the root hair with a decrease in temperature of  $5\frac{1}{2}$  degrees (from  $26.5^{\circ}$  to  $21^{\circ}$ ). Later, elongation was resumed intermittently, but with a return to normal room temperature the growth was extremely slow.

Nos. 50 and 51 show a reduction in the growth rate to zero in from 10 to 20 minutes, with a temperature reduction of only  $1\frac{1}{2}$  to 2 degrees.

No. 52 shows the effect of an increase of temperature from  $25^{\circ}$  to  $32.5^{\circ}$  C. The growth rate was first retarded and then increased 14 microns over that before heating. However, at a continuous temperature of  $32^{\circ}$ , the root hair became swollen at the tip and no further growth was noted.

No. 53 shows a less decided reaction with an increase in temperature from  $25^{\circ}$  to  $26^{\circ}$  C. There was first a retardation and then an acceleration in growth. The acceleration here was slight, and growth soon returned to that found before heating. As the temperature increased to  $28^{\circ}$ , a second acceleration was noted.



TEXT FIG. 6. Nos. 50 and 51 show the effect of a cooling solution on root hairs of radish. No. 52 shows the effect of a heating solution on a root hair of white mustard; Nos. 53 and 54 show the effect of a similar solution on root hairs of radish. For discussion see text.

No. 54 shows an acceleration in growth with an increase in temperature of  $3^{\circ}$  C. Two applications of heat were made, the growth rate increasing in both cases during heating and dropping to normal shortly after heat was removed. The return of the growth rate to that found before heating took place more rapidly than the drop of temperature. No swelling was noted in this hair.

These investigations indicate that the root hair is extremely sensitive to changes of temperature when grown within the limits of  $17^{\circ}$  to  $27^{\circ}$  C. A drop in temperature taking place within a period of 5 to 15 minutes results in

a decrease in the growth rate. In some cases recovery is partial or even entire. In other cases elongation ceases and is not resumed. A reduction in temperature of 5 to 6 degrees results in a contraction of the root hair. With a reduction of one to two degrees no shortening is noted, although elongation may be entirely stopped. This shortening may be the result of a turgor change within the root-hair cell.

A gradual increase in temperature of  $2\frac{1}{2}$  to 3 degrees results in a temporary acceleration of growth, which, however, soon returns to normal. This return to normal rate may take place more rapidly than the return to room temperature. An increase in temperature of from 3 to 6 degrees results in swelling of the root-hair tip and cessation of growth. In some cases growth may be resumed later. It was previously noted that growth under high light intensities, where the temperature factor could not be entirely eliminated, resulted in such a swelling as described.

The work of previous investigators has been with multicellular organs (mostly the radicles or young shoots of seedlings) over one or more days with readings in most cases one half to three hours apart. Their variations in temperature have also been much greater than those used in the present investigations. They failed to find any marked effect on growth in consequence of such slight temperature changes as produced a decided reaction in the root hair. This failure may be due, in part at least, to the fact that the cells making up complex tissues are more protected from sudden changes of temperature. Thus, in the root, the epidermal cells and those of the outer cortical region protect the internal cells from sudden changes of temperature. On the other hand, the long slender root hair (exclusive of the basal portion of the epidermal cell from which it arises) is directly exposed on all sides to the varying temperature changes of the surrounding atmosphere. In spite of this difference there is some similarity in results. Thus, Godlewski (16) and True (45) found that a lowering of temperature resulted in a temporary reduction in the growth rate. True, in the case of *Vicia faba* already mentioned, found, when the radicle was subjected to suddenly lowered temperature, that not only a cessation of growth but actual contraction followed. He used very low temperatures, however ( $0^{\circ}$  C.), and found little effect of temperatures from  $18^{\circ}$  to  $30^{\circ}$  C. Askenasy (2) found that an increase in temperature, when the root was grown near the optimum, resulted in an increase in the growth rate.

The effect of temperature on the elongation of the root hair may be an effect either on the nature of the plasma membrane, on the osmotic pressure or colloidal imbibition of the cell contents, or on the extension of the cell wall. Livingston (30) states that a potent cause for great increase in protoplasmic permeability in some instances is a lowering of temperature. Thus, if a filament of a common alga is carefully dried and placed in olive oil, whose temperature is then rapidly lowered, a film of water may be seen to form about the filament and partial plasmolysis may be observed.

When the temperature is again brought back to normal, the extruded water is absorbed. Greely (17) has shown that complete plasmolysis can be produced in *Spirogyra* by lowered temperature.

The increase in cell permeability due to lowered temperature may account for the decrease in elongation in certain of the root hairs studied. Partial plasmolysis in the root hairs would doubtless result in the cessation of elongation and even in contraction. The growing root hair being under tension, a stoppage of the growth processes with lowered osmotic pressure would, if sufficiently marked, result in a contraction and shortening of the wall due to its elasticity.

Krabbe (24), experimenting upon the effect of a rise in temperature upon the absorption of water by various plant cells, found the rate of absorption to rise with the temperature. Ursprung (46) found with shoots of *Fagus* and *Thuja* that an increase in the temperature of the water in which the shoots were immersed was followed at first by no increase in the water-absorption. Later there was a sudden increase in absorption, and finally a decrease to the zero point. Van Rysselberghe (47) and Brown and Worley are others who have worked on the relation of temperature to the permeability of protoplasm and the ability of tissues to absorb water.

The effect of changes of temperature on the osmotic pressure of various colloids has been studied with varied results. Moore and Roaf (32) found that the osmotic pressure of gelatin solutions increases faster than the absolute temperature. Recently, C. R. Smith (43) has studied the variations in osmotic pressure of gelatin in water. He concludes that "the swelling of gelatin is the result of osmotic pressure within the jelly, with the jelly acting as an imperfectly resisting membrane, the more so when highly swollen." This indicates that the effect of temperature upon the osmotic pressure of the cell may be practically identical with its effect upon colloidal imbibition. Shull and Shull (41) have found that gelatin absorbs water for a long period from a saturated atmosphere—in fact for a much longer period (47 days) than these root hairs continued to absorb water. It may be, therefore, that the whole process of root-hair elongation is primarily a matter of colloidal imbibition, with osmotic pressure and cell-wall deposition as secondary. Lillie (29) noted interesting thermal after-effects, or so-called hysteresis phenomena, in gelatin solutions. These, when heated and afterward cooled, continued for some time to show a higher osmotic pressure than when kept continuously at the lower temperature.

Further evidence of the importance of the effect of temperature on colloidal imbibition in connection with the growth of these root hairs is shown by the swelling of their tips with sudden changes in temperature. This swelling must involve a change in the cell wall as well as in the interior of the cell. Recent evidence indicates that the cell wall is colloidal in nature, and the swelling of the root hair above described contributes to the support of this idea.

## SUMMARY

1. The grand period of elongation of the root hair resembles the grand period of growth in plant tissues in general, except that the middle of the curve is more extensively flattened in the case of the root hair. There is a gradual increase in the growth rate during the early part of the period and a gradual diminution in rate at the close.

2. There is little difference in the growth rate of root hairs on any one root. Slight differences in length of root hairs in any one region are due more to age than to rate of growth.

3. There appears to be a definite relation between number of root hairs and rate of growth. On species having many root hairs, the hairs elongate more slowly than on those with fewer hairs.

4. There is a definite relation between root-hair elongation and lateral movement, the rate of elongation being gradually accelerated as the lateral movement of the hair is retarded. Apparently cellular interaction is a factor here. The checking of cell-elongation in the epidermis due to a decrease in the rate of growth in the adjacent cortical cells results in an evagination in certain epidermal cells which develop root hairs.

5. Root hairs of *Raphanus sativus* and *Sinapis alba* show no noticeable light-growth reaction in light intensities up to 3153600 M. C. S., and the latter species shows no such reaction in continuous lighting for two hours under an intensity of 938 M. C. It is therefore suggested that the light-growth reaction in multicellular organs is not due to a change in the rate of cell-elongation, but to some other effect of light, such as a change in the rate of cell-division.

6. Root hairs of *Raphanus sativus* and *Sinapis alba* grown at a constant temperature within the limits of 17° and 27° C., show a decided reaction to changes of temperature of from one to six degrees brought about within a period of from 5 to 15 minutes. A drop in temperature results in a decrease in the growth rate and sometimes even in contraction of the root hair with no indication of swelling. In some cases recovery of the growth rate is partial or even entire; in other cases elongation ceases and is not resumed. A rise in temperature of 2½ to 3 degrees in the time given above results in a temporary increase in the growth rate, which soon returns to that before heating. The return to what we may call the normal rate often takes place while the temperature of the surrounding air is still above normal. These results indicate that a change in temperature affects the rate of growth in multicellular organisms by modifying the rate of cell-elongation.

7. It is concluded that the effect of temperature upon the rate of growth of tissues is in part at least an effect upon the osmotic pressure and imbibition of the cell colloids, rather than simply an effect upon the rate of chemical reactions within the tissues.

This study was carried out in the laboratory of plant physiology at the University of Iowa, and the writer wishes to express his appreciation to

Dr. C. H. Farr, who suggested the problem, and whose constant interest and helpful suggestions have been invaluable aids in the completion of the experiments.

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#### EXPLANATION OF PLATE LXII

- FIG. 1. Moist chamber containing radish seedlings.
- FIG. 2. Temperature chamber used in investigating the effect of varying temperatures on the rate of root-hair elongation.
- FIG. 3. Photograph showing swelling of root hairs due to increased temperature. These hairs continued to elongate after swelling.
- FIG. 4. Border of radish root in the root-hair zone. Photographed at 15-minute intervals (right to left). Note the lateral (downward) movement of the young root hairs shown in the lower portion of the photograph.
- FIG. 5. Photograph of radish root in the root-hair zone.

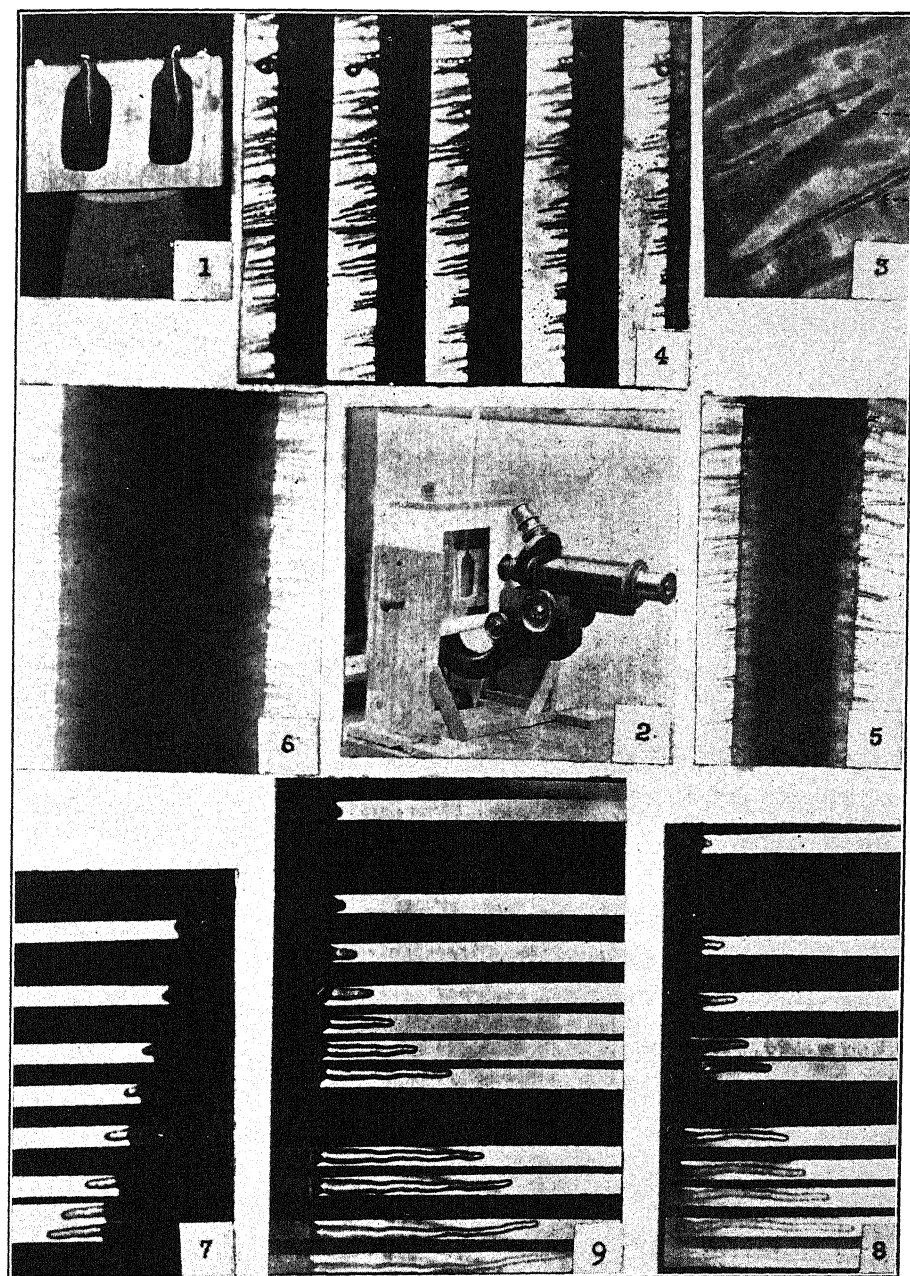
FIG. 6. Photograph of pop-corn root in the root-hair zone.

FIG. 7. Root hair of pop corn photographed at 15-minute intervals for one hour and forty-five minutes. Spaces between the photographs show the amount of lateral (downward) movement during each interval. Note the decrease in the rate of root-elongation with the increased lengthening of the root hair.

FIG. 8. Root hair of pop corn photographed at 15-minute intervals. Spaces between the upper five photographs show lateral movement. The lower five show root-hair elongation only.

FIG. 9. Root hair of radish photographed at 15-minute intervals for  $2\frac{1}{2}$  hours. The spaces between the upper seven photographs show the amount of lateral movement during each interval. The lower four photographs show root-hair elongation only.





JEFFS: ELONGATION OF ROOT HAIRS



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## GREENSAND AS A SOURCE OF POTASSIUM FOR GREEN PLANTS

JOHN ROBSIN SKEEN

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### HISTORICAL INTRODUCTION

The history of marl<sup>1</sup> in America antedates the Revolution. It appears to have been discovered in 1768 near Marlboro, New Jersey, on the farm of Peter Schenk (3). The large crops harvested on the land treated with this material led to its local use. However, it was not until about 1817 that its employment was general. In 1818, Edmund Ruffin (10) discovered a like deposit on the James River below Richmond, Virginia. He began perhaps the most careful and thorough series of practical tests to which the marls were put in the early decades.

Henry Seybert (11), a chemist in Philadelphia, demonstrated the presence of potassium in samples of greensand from New Jersey in 1822. At that time it was not known that potassium was essential for plant growth. Not until 1835 did Professor William B. Rogers (9), then of William and Mary College, point out the probable importance of this element as analyzed from the greensands.

The early farmers of New Jersey were aware of the benefits to be derived from marling. George Craft (4) and Mark Reeves (8) both reported favorably as early as 1818. George H. Cook (3) in 1868 writes that ". . . marl . . . has been of incalculable value to the country in which it is found. It has raised it from the lowest stage of agricultural exhaustion to a high state of improvement." Continued good reports supported the use of marl, and much of southern New Jersey was saved from depopulation by its benefits.

The application of greensands in Virginia was widely practiced before the Civil War with generally favorable results. Along the courses of the James, Pamunkey, Mattaponi, and Rappahannock Rivers, the digging of

<sup>1</sup> The word "marl" is used to designate a deposit in which calcium carbonate, often in the form of shells, is the important constituent. When the calcareous component is small or practically lacking, and the major constituent is glauconite, the term "greensand" is used.

marl in the winter became an essential part of the farm routine. The effects of marling as reported in the old writings were evident from ten to thirty years after its application.

Marling was deemed good farm practice until the early 50's, when the quickly available guanos were introduced. Their cheap price and startling results were impressive, and by their use the drain on labor necessitated by the digging of marl was obviated. Then, too, the use of phosphates in soils deficient in them overshadowed the effects produced by marl. The readily soluble nitrates from Chile obtained a vogue about this time, followed by the concentrated potash fertilizers from the mines of Stassfurt, Germany. Marl could not at that time compete in cost and in immediate efficiency with such fertilizers, and it rapidly fell into disuse. Its value has since nearly been forgotten.

#### LOCATION AND NATURE OF GREENSANDS AND MARLS

In a general way, it may be said that these substances are found from northern New Jersey to Georgia, east of the Fall Line. Their exact location and extent have been ascertained in New Jersey and Virginia (3, 6, 9). In the former state, there is a particularly rich belt extending from the vicinity of Salem on the Delaware River northeast to the region around Long Branch. The belt marks a serpentine course and varies in width from less than a mile to more than fifteen miles. The best deposits in Virginia have been located along the courses of the rivers. Less definite information apparently is available as to the location of marl pits in Maryland and Delaware. Clark (2) gives a clear account of the geologic location in these states.

No marl or greensand is present in the lower Cretaceous. The upper Cretaceous, unknown in Virginia and receiving its clearest expression in New Jersey, is present to some extent in Maryland and Delaware. The sands of this formation are often more or less glauconitic, frequently highly calcareous. The Eocene in Maryland overlies the Upper Cretaceous, while in Virginia it has transgressed the latter and is found overlying the Lower Cretaceous. The deposits in Maryland (2) are chiefly of greensands, either calcareous or argillaceous. The Miocene deposits attain an extensive development in the drainage basin of Chesapeake Bay in both Maryland and Virginia. Numerous marl beds are present which are packed with molluscan shell remains. Marls for the most part are a product of the Miocene and lie at varying distances from the surface. In descending order, the following formations may present themselves: (1) White, friable, sandy clay containing fossil impressions. (2) White, sandy marl with broken shells. (3) Ferruginous stratum, generally with some shells. (4) A thin band of black pebbles. Such deposits are shown by analysis to contain from 25 to 95% calcium carbonate (9). The percentage of green or black particles is necessarily small.

The Eocene underlies the Miocene, and the following layers of varying thickness may be discerned: (1) Dark greensand stratum, no shells. (2) Layer of cemented shells of the saddle-shaped oyster. (3) Dark greensand stratum with small shells.

From the above description, the significance of the words "marl" and "greensand" is apparent. Greensands, characterized by glauconite, belong to the Eocene, never to the Miocene or Upper Cretaceous; marls, characterized by a high content of  $\text{CaCO}_3$ , may belong to all three, but generally occur in the Miocene.

Greensand is composed largely of glauconite, small rounded particles which are complex and rather stable silicates. True and Geise (12) and Kelly (5) analyzed samples from New Jersey and Virginia and found varying amounts of S, Ca, Mg, P, Na, and K. Mansfield (6) has made a mechanical analysis of the greensands and marls of New Jersey. Blair (1) has analyzed a number of New Jersey marls and greensands for phosphoric acid and potash. The beds are unconsolidated deposits of the greenish grains, clay, quartz, and other minerals, of no specific composition.

#### OBJECT OF THE EXPERIMENTS

Dr. Rodney H. True, under whose supervision this work was prosecuted, suggested the experiments as a continuation of the work done by him in collaboration with Geise (12). They performed a series of experiments on wheat and clover, using greensands from "Courtland," near Hanover Court House, Virginia, and from Red Bank, New Jersey, and marls from Pamunkey Valley, Virginia, and from Red Bank, New Jersey.

The criterion of the availability of the potassium from the greensands and marls used was the weight of the air-dried tops. The results were conclusive so far as they went, but there was some doubt as to whether appreciable differences in the potassium content would be apparent upon a further analysis of the cultures. It was to ascertain this point among others that the present work was undertaken.

#### METHODS

Three series of experiments were conducted simultaneously:

- (A) Six pots prepared with no greensand added and no potassium as the nitrate.
- (B) Thirty pots with no potassium added as the nitrate, but with the addition of greensand in the following amounts:
  - (1) Six pots with  $\frac{1}{2}\%$ .
  - (2) Six pots with  $\frac{3}{4}\%$ .
  - (3) Six pots with 1%.
  - (4) Six pots with 2%.
  - (5) Six pots with 4% greensand added.
- (C) Six pots with no greensand, but with potassium added as the nitrate.

Series *A* and *B* are immediately comparable as to the benefits derived from the greensand used as a source of potassium for the plants. Series *C* is here considered as an optimum.

Glazed porcelain pots eight inches in depth were employed. These were filled nearly to the top with Juniata crushed quartz. In series *B*, the sand was thoroughly mixed with the appropriate amounts of greensand. The sand was first well washed with dilute HCl, with tap water, and finally with distilled water. The greensand was obtained from Marlboro, New Jersey, and contained 5.8% potassium. Marquis variety of spring wheat was used as the test plant, and 20 seeds were planted to each pot January 27, 1923.

It is well known that potassium absorption is greatest when increase in volume is the predominant feature in plant development, that is, during the early stages of growth. For this reason, the plants were not carried to maturity. They were harvested March 12, after a growth period of a trifle more than six weeks.

Series *A* and *B* were fed the same nutrient, made up as follows:

Potassium nitrate.....	0.00000 M.
Phosphoric acid.....	0.00050 M.
Calcium nitrate.....	0.00306 M.
Magnesium sulfate.....	0.00018 M.

Series *C* was given the following:

Potassium nitrate.....	0.00191 M.
Phosphoric acid.....	0.00050 M.
Calcium nitrate.....	0.00203 M.
Magnesium sulfate.....	0.00030 M.

The osmotic pressure of the nutrients was something under 0.1 atmosphere. No provision was made for aëration or drainage, and it was necessary to add the nutrients in dilute amounts to insure an osmotic surplus for the plants at the conclusion of the experiments. The comparatively large amounts of calcium were used in order to make the potassium easily available and to furnish a neutralizing agent for toxic salts excreted by the roots. Since wheat will tolerate a considerable range of acidity, it was not thought necessary to control the pH of the solutions.

Great care was taken in harvesting to obtain the entire root system. The harvest of each pot was kept together and thoroughly washed to remove sand and greensand. All the greensand was removed, but it was found impracticable to rid the roots of all the sand. The green weight was recorded as "green weight plus sand." The plants were then stuffed into tared test tubes and dried for 24 hours in an oven regulated at 95° C. The "dry weight plus sand" was recorded. The harvest of each pot was then charred in a platinum dish and water-soluble salts were extracted. Determinations for ash and sand followed. Total alkali chlorids and potassium were determined by the methods recommended by the Association of Official Agricultural Chemists (7).

## RESULTS

No difference was apparent in the cultures until about two weeks after planting. Series *C*, and those cultures of series *B* in which 2 and 4% greensand were used respectively, appeared slightly larger than the others. In three weeks this difference was well marked, and series *A* began to show the stunting effect of potassium starvation. The comparison at harvest is best shown by the photograph of text figure 4. Two representative pots of each treatment are pictured. Reading from left to right in pairs are the pots treated with 4%, 2%, 1%,  $\frac{3}{4}$ %,  $\frac{1}{2}$ % greensand, with potassium nitrate, and with no potassium and no greensand. The differences shown in the tops need no comment.

Table 1 lists results of analyses of the entire plant reduced to a percentage basis. The values of series *C* in all cases are used as 100%, and the actual values of these analyses may be found at the heads of the columns, listed as the values for potassium nitrate. Further, each analysis is reported as the weight per plant. It was thought best to report them in this manner for the sake of a more direct comparison.

As was to be expected, varying numbers of plants were matured in the pots. For this reason the pot analysis is not comparative. Then, too, the pots in which large numbers of plants matured, although having a greater total weight of plants, had a less weight per plant than those pots in which few plants matured. Thus it became necessary to accept results of pots producing the same number of plants. Nineteen pots only, of the total of forty-two, are therefore available as criteria.

Table 2 sums up the potassium relationships, and is designed to give a comparison of the efficiency of the various percentages of greensand used with that of potassium nitrate.

Text figures 1 and 2 show graphically the results summarized in table 1, and text figure 3, the percentage of potassium extracted per pot from the total amounts available, as reported in table 2.

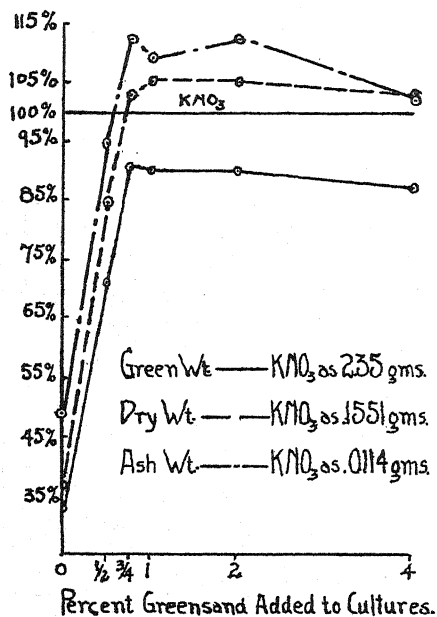
## DISCUSSION

The large variations in results in pots treated in exactly the same manner and harvesting the same number of plants forbid the acceptance of the results as precise determinations. There are three errors to be considered: First, the biologic variation, which is assuredly large even under the best of circumstances; second, the personal equation in the making of determinations and in technique; and third, the cumulative errors arising from making all determinations from the same initial sample.

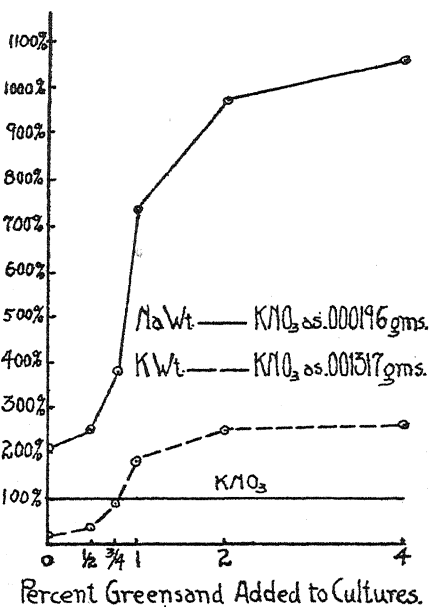
The variations in green weight are due for the most part to variations in cultures and to the loss of varying amounts of roots in harvesting. The loss of root substance is not so apparent in the dry weights. Ash and sand were determined in fused silica crucibles. Such crucibles lose more or less upon prolonged heating, so that, even though the losses were corrected

as closely as possible, they are nevertheless the source of considerable error, as is apparent from table I.

The Na and K determinations are thought to be entirely reliable within the limits of the initial variation.



Text Fig. 1: Graph showing analyses of all cultures compared to  $KNO_3$  as 100%.



Text Fig. 2: Graph showing Na and K absorption in all cultures compared to  $KNO_3$  as 100%.

TEXT FIG. 1. Graph showing analyses of all cultures for green weight, dry weight, and ash weight compared to  $KNO_3$  as 100%. TEXT FIG. 2. Graph showing analyses of Na and K absorbed by all cultures compared to  $KNO_3$  as 100%.

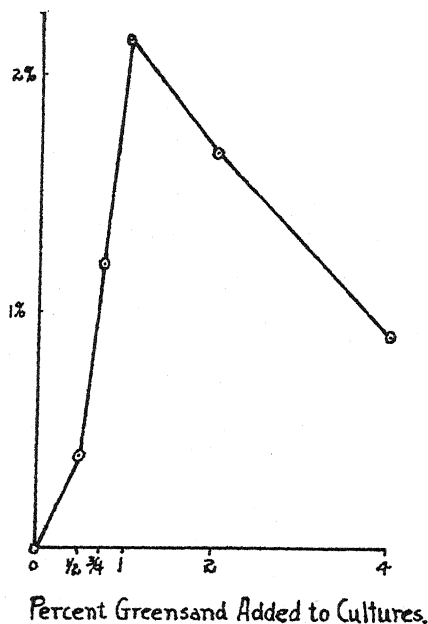
The increase in percentage weights of series B over series C in dry and ash weights may be attributed to some extent to the absorption of the heavy metals from the greensand. Under the conditions of the experiments, the addition of 4% greensand apparently has a toxic effect on the plants. It is probable that if more calcium had been used in the nutrient this effect would have been entirely obviated. The value of the tangent of all the curves of text figure 1 may be considered as zero with the addition of  $\frac{3}{4}$ % greensand. In so far as the economic value of wheat (before heading out) depends on the green and dry weight of the plant, only the results shown in text figure 1 are accepted as criteria for the benefits to be derived from greensand as a source of potassium for plants.

It will be noted that the yield is not materially increased by any addition of greensand in excess of  $\frac{3}{4}$ %. Under the conditions cited, this amount is



considered the best economically. Calculated to the surface 8 inches of sand, such an addition is equivalent to a trifle more than 11 tons per acre. Additions equivalent to 40 tons per acre show practically the same effect.

Text figure 2 shows that the sodium and potassium from the greensand are entirely available for plant use, and that the maximum absorption is attained by those plants growing in 2% greensand.



Text Fig. 3: Percent K Present in  
Culture Medium Ab-  
sorbed by Plants.

TEXT FIG. 3. Percentage of K present in culture medium absorbed by plants.

In series *C* (as shown by table 2) only 13% of the potassium added was extracted by the plants. This indicates that a large excess of potassium was present in the pots of this series, but that the plants had no need for the excess. Knowing that the potassium in greensands is tied up as a rather difficultly soluble silicate (5, 6, 12), the results shown in text figure 2 are very confusing. If series *C* absorbed only so much potassium despite the excess, why also should not series *B* absorb an amount approaching that of *C* as a limit? An increase of 155% seen in the better greensand cultures is not explained by table 2, which indicates much larger amounts of potassium present as an excess in series *B* than in series *C*. It is not conceivable that mere mass of material could have such an effect, particularly when it is known that that material is not nearly as soluble as potassium nitrate.

TABLE 1. *Analyses of Wheat Plants Grown in Sand Cultures with Greensand and Potassium Nitrate as Potassium Sources*

Potassium Source	Number of Pots Accepted	Number of Plants	Green Weight Compared to $\text{KNO}_3$ 2.35 g.	Variation and Error	Dry Weight Compared to $\text{KNO}_3$ 0.1551 g.	Variation and Error	Ash Weight Compared to $\text{KNO}_3$ 0.0114 g.	Variation and Error	Na Weight Compared to $\text{KNO}_3$ 0.000196 g.	K Weight Compared to $\text{KNO}_3$ 0.001317 g.	
A None..	2	28	% 33.6	% 10.0	% 36.5	% 9	% 49.1	% 15	% 213.0	% 24	
Greensand B	$\frac{1}{2}$ %....	3	45	71.1	2.5	84.7	3.5	94.7	14	263.0	37
	$\frac{3}{4}$ %....	3	43	90.4	1.5	102.9	2.4	112	4	377.0	92
	1%....	2	29	90.0	8.0	105.0	3.0	108.8	10	735.0	180
	2%....	3	43	90.2	1.5	105.0	2.8	112.2	11	987.0	235
	4%....	3	45	86.8	.9	103.0	2.5	102.6	7	1077.0	255
C $\text{KNO}_3$ .	3	44	100	5.9	100	5.0	100	10%	100	100	

Text figure 3 shows the relative degree of availability of the potassium as a function of the total amount added. Beyond a doubt, the plants most efficiently remove potassium in the experiments from the pots with one percent greensand added. However, text figure 1 shows no increase in green, dry, or ash weight over the production with  $\frac{3}{4}$ % greensand, therefore such an amount apparently is not economically the best to add.

TABLE 2. *Potassium Absorption by Wheat Plants in Sand Cultures with Greensand and Potassium Nitrate as Potassium Sources*

Potassium Source	Grams Green-sand Added per Pot	Grams K Added per Pot	Grams K Harvested per Pot	Grams K Extracted from Nutrient	Percent K Extracted per Pot	
A None . . . .	00.00	0.00	.0045	.0000	0.00	
Greensand B $\frac{1}{2}$ % . . . . .	12.00	0.696	.0073	.0028	0.40	
	$\frac{3}{4}$ % . . . . .	18.00	1.044	.0170	.0125	1.19
	1% . . . . .	24.00	1.392	.0345	.0300	2.15
	2% . . . . .	48.00	2.784	.0511	.0466	1.67
	4% . . . . .	96.00	5.568	.0555	.0510	0.91
C KNO <sub>3</sub> . . . .	00.00	0.1103	.0191	.0146	13.2	

Practically no more potassium is absorbed by the plants growing in the 4% greensand than by those in 2%, the excess remaining available but unused. This point is brought out in text figure 3.

### CONCLUSIONS

1. Greensands are a physiologically available source of potassium for plant growth.

2. The percentage of potassium in the greensand used for plant growth depends upon the quantity of greensand added. The percentage increases in a sharply linear curve up to probably 2% and falls away sharply upon the addition of more greensand.



TEXT FIG. 4. Reading from left to right, cultures in pairs containing 4%, 2%, 1%,  $\frac{1}{2}$ %,  $\frac{1}{4}$ % glauconite,  $\text{KNO}_3$ , and no added source of K.

3. No loss of potassium results from the addition of great amounts of greensand. Plants evidently have a maximum quantity which they can absorb. The unabsorbed amounts, glauconite being only minutely soluble in water, remain in the soil until needed.

4. The average potassium-short soil should show marked improvement for several years from the addition of from 5 to 15 tons of greensand per acre. In using greensands, it is best to have considerable calcium present either in the soil or composted with the greensand in order to obviate any toxic effect that may be brought about by a low pH value.

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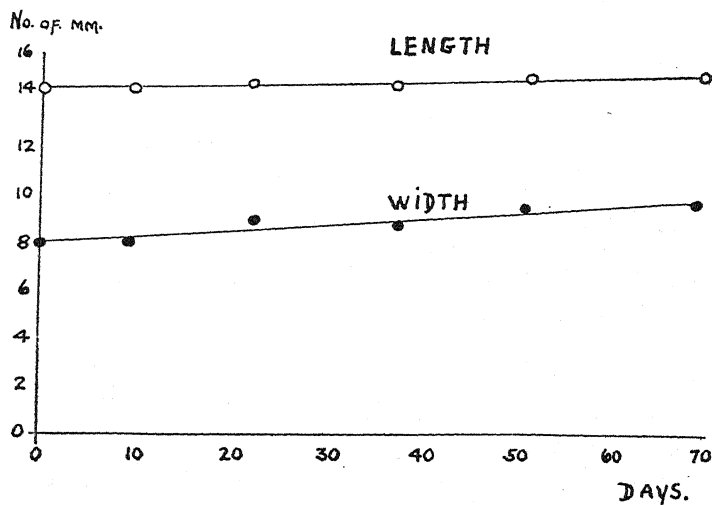
# A NOTE ON THE RATE OF GROWTH OF *VALONIA MACROPHYSA*

MATILDA MOLDENHAUER BROOKS

(Received for publication December 26, 1924)

In order to determine the length of time required for *Valonia macrophysa* to attain a usable size, the rate of growth of this plant under laboratory conditions was measured.

The plants grow in clusters and are usually elongated in shape or compressed in one direction like palisade cells or the cells of most epithelia. For this experiment, the clusters were broken up into their individual cells and each cell was placed in a 200-cc. glass beaker containing sea water which was renewed every day. It was necessary to keep these in a shaded part of the room, as direct sunlight eventually cytolyzes the cells.



TEXT FIG. 1. Growth curves of *Valonia macrophysa*.

The diameters and lengths of the cells were measured by allowing them to pass through circular holes made in a piece of cardboard. These holes were graduated from 5 mm. to 20 mm. in steps of 1 mm. Measurements were taken between May 17 and July 25 after intervals of 9, 22, 37, 51, and 68 days. The temperature in the room did not vary greatly from 22° C. during this time.

## RESULTS

The averages of the measurements of 32 cells at the time intervals specified are given in table 1.

TABLE 1. *Measurement in mm. of Valonia macrophysa (Average of 32 Cells)*

Days.....	0	9	22	37	51	68	Total Gain in Mm.
Width.....	8	8	9	8.8	9.7	9.8	1.8
Length.....	14.3	14.5	14.9	14.9	15.1	15.2	.9

Text-figure 1 shows that gain in both width and length is a slow process. The gain in width was greater than that in length, being 1.8 mm. for the entire time as compared with 0.9 mm.

The shape of this plant is influenced by crowding and other conditions which evidently account for its deviation from sphericity. When these conditions are not present, as, for example, when the cells are separated and placed individually in glass dishes of sea water, the cells tend to assume a spherical shape as rapidly as the necessity for building new cell wall will permit. This accounts for the greater increase in the diameter of the cells as compared with that in length.

I am indebted to Miss Eleanor M. Steele for making these measurements.

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COMPARATIVE STUDIES ON RESPIRATION XXVIII.  
THE EFFECT OF ANESTHETICS ON THE PRO-  
DUCTION OF CARBON DIOXID BY WHEAT  
UNDER AËROBIC AND ANAËROBIC  
CONDITIONS

ASTRID KARLSEN

(Received for publication January 7, 1925)

The relation between the aërobic and the anaërobic production of carbon dioxid has been the subject of much discussion and is still far from settled. It would seem that the problem can be attacked by comparing the aërobic and the anaërobic behavior of the same organism under a variety of conditions which affect respiration. If the aërobic and the anaërobic methods of production of carbon dioxid are essentially alike, they should vary in similar fashion when subjected to such influences. But if they are fundamentally different, this fact should become clear when a sufficient variety of conditions has been studied.

No quantitative studies of this kind have yet been made, so far as the writer is aware, with the exception of a recent paper by Lyon.<sup>1</sup> The present paper describes a quantitative investigation of the effect of certain anesthetics upon the production of carbon dioxid by wheat. No attempt has been made to investigate the subject in all its bearings, since the purpose was merely to make certain exploratory experiments in order to discover whether any striking differences exist in the behavior toward reagents under aërobic and anaërobic conditions.

The method employed is that described by Osterhout.<sup>2</sup> In the anaërobic experiments the oxygen was driven out of the apparatus as far as possible by means of a current of nitrogen (from a cylinder of compressed nitrogen). Before entering the apparatus it passed in succession through a series of towers containing  $(\text{NH}_4)_2\text{CO}_3$ , concentrated  $\text{NH}_4\text{OH}$  plus copper gauze, and dilute  $\text{H}_2\text{SO}_4$ . The current of nitrogen was allowed to flow through the apparatus for half an hour before starting the experiment. A bottle of alkaline pyrogallol was placed in the apparatus; through this the circulating gas constantly bubbled. If it became so dark as to indicate leakage of oxygen from outside, the experiment was rejected. Not infrequently there was some darkening during the course of an experiment, indicating the presence of traces of oxygen, which, however, were so promptly removed by the pyrogallol that they did not appear to interfere with the results.<sup>3</sup>

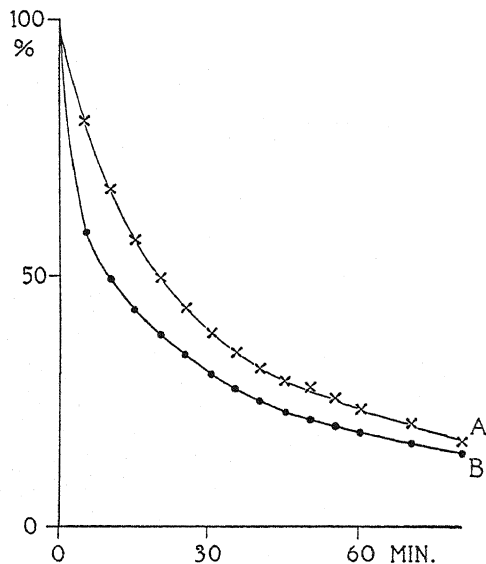
<sup>1</sup> Lyon, C. J. Jour. Gen. Physiol. 6: 299. 1924.

<sup>2</sup> Osterhout, W. J. V. Jour. Gen. Physiol. 1: 17, 171. 1918-1919. 2: 1. 1919-1920.

<sup>3</sup> There was so little leakage that the empty apparatus (containing no organisms) would run for half an hour with practically no change of color in the indicator.

Since the seedlings were moist and the indicator tube contained water, the circulating current of gas was always moist.

In preparing an experiment the dry seeds were put for half an hour in commercial hydrogen peroxid (3%  $\text{H}_2\text{O}_2$ ) and then placed on moist filter paper in sterile petri dishes. When the roots were 2.5 to 3 cm. long, the seedlings were removed for use. Absence of mold was regarded as indicating that the seedlings were reasonably free from contamination by bacteria. Usually 100 seedlings were put for ten minutes or more in running water to remove accumulated carbon dioxid and then placed in the respiration chamber.



TEXT FIG. 1. Rate of production of carbon dioxid by wheat: *A* in air saturated with ether vapor, *B* in nitrogen saturated with ether vapor. In curve *A* the rate is expressed as percentage of the normal rate in air, in curve *B* as percentage of the normal rate in nitrogen (this is about 60% of the normal rate in air). Curve *A*, average of 4 experiments, curve *B*, average of 5 experiments. Probable error of the mean less than 10% of the mean.

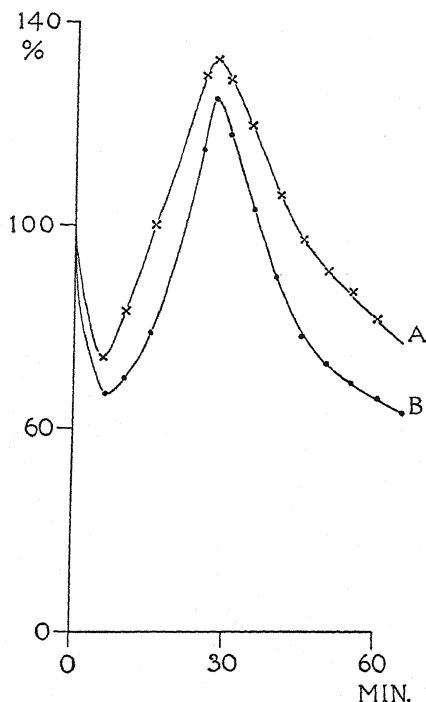
When the normal rate of respiration had become constant, the reagent was introduced into the bottom of the respiration chamber by means of a separatory funnel in such a way that no air was admitted from outside. The seedlings were supported on glass rods in the chamber so that they did not touch the bottom and hence did not come in direct contact with the reagent, which acted on them only in the form of vapor. All the substances employed were very volatile, and their vapor was quickly carried through the apparatus by the circulating current of gas which bubbled through the reagent.

The temperature averaged about 20° C. Ordinarily the variation during an experiment did not exceed 1° C. (in a few cases it amounted to 2° C.).



In order to compare the rate under aërobic and anaërobic conditions, several lots of seedlings were tested. The aërobic rate was first measured. When it had become constant, the oxygen was driven out of the apparatus by means of a current of nitrogen (without removing the seedlings), and the anaërobic rate was then measured in the same manner. It proved to be quite consistently about 60% of the aërobic. (Lyon<sup>4</sup> reports it to be 72%.)

In all the figures the rate is expressed as percentage of the normal. The normal rate is the reciprocal of the time required to change the pH value in the indicator tube (phenolesulphonephthalein) from 7.78 to 7.36. This time was ordinarily about 60 seconds for aërobic and about 100 seconds for anaërobic experiments.

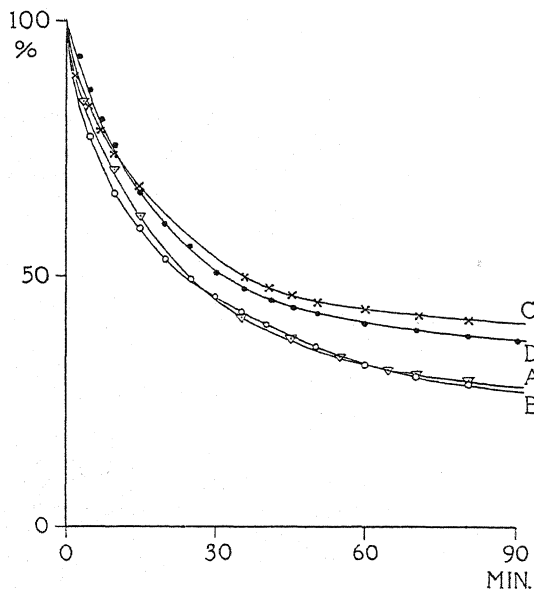


TEXT FIG. 2. Rate of production of carbon dioxide by wheat: *A* in air partially saturated with ether vapor, *B* in nitrogen partially saturated with ether vapor. In curve *A* the rate is expressed as percentage of the normal rate in air, in curve *B* as percentage of the normal rate in nitrogen (this is about 60% of the normal rate in air). Each curve represents a single typical experiment.

Text figure 1 shows the results obtained by introducing sufficient ether (10 cc.) into the respiration chamber to saturate the air in the apparatus with ether vapor. Curve *A* shows the rate of production of carbon dioxide

<sup>4</sup> *Loc. cit.*

in air, expressed as percentage of the normal rate, which was ascertained before introducing the ether. It will be seen that as soon as the ether is introduced the rate begins to fall and continues to do so in a regular manner during the course of the experiment. Curve *B* shows the behavior in nitrogen saturated in the same manner with ether vapor. Since the rate in curve *B* is expressed as percentage of the normal rate in nitrogen (before the introduction of ether), it begins at 100%, as does curve *A*, and is therefore easily compared with it.

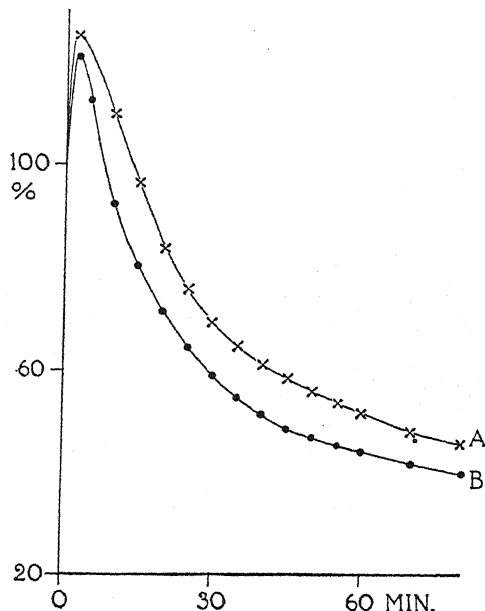


TEXT FIG. 3. Rate of production of carbon dioxide by wheat: *A* in air saturated with benzene, *B* in nitrogen saturated with benzene, *C* in air partially saturated with benzene, *D* in nitrogen partially saturated with benzene. In curves *A* and *C* the rate is expressed as percentage of the normal rate in air, in curves *B* and *D* as percentage of the normal rate in nitrogen (this is about 60% of the normal rate in air). Curves *A* and *B*, averages of 5 experiments each; curve *C*, average of 4 experiments; curve *D*, average of 3 experiments. Probable error of the mean less than 9% of the mean, except at one point on curve *A*, where it is 12% of the mean.

It will be seen that the effect of ether is very similar whether the experiment is conducted in air or in nitrogen. This is also true when we use a smaller amount of ether (1 cc.) so that the gas in the apparatus does not become saturated with ether vapor. The curves, however, differ from those shown in text figure 1; the rate falls, then rises above the normal, reaches a maximum, and again begins to fall, as shown in text figure 2. These curves recall those of Miss Irwin,<sup>5</sup> who found that in certain animals ether may cause a decrease in the output of carbon dioxide; this is followed

<sup>5</sup> Irwin, M. Jour. Gen. Physiol. 1: 209. 1918.

by an increase, after which the rate falls off. These curves obtained by the writer are very similar to those obtained by Miss Smith<sup>6</sup> in studying the effect of ether on the production of carbon dioxide by wheat.



TEXT FIG. 4. Rate of production of carbon dioxide by wheat: *A* in air containing alcohol vapor, *B* in nitrogen containing alcohol vapor. In curve *A* the rate is expressed as percentage of the normal rate in air, in curve *B* as percentage of the normal rate in nitrogen (this is about 60% of the normal rate in air). Curve *A*, average of 4 experiments; curve *B*, average of 3 experiments. Probable error of the mean less than 7% of the mean.

Text figure 3 shows the effect of benzene. When a sufficient amount (5 cc.) is introduced to saturate the contents of the apparatus, we obtain curves *A* (aërobic) and *B* (anaërobic) which are practically identical. When a smaller amount (1.3 cc.) is used, so that the contents of the apparatus are not saturated with the vapor, we obtain curves *C* (aërobic), and *D* (anaërobic), which are also in close agreement.

It seemed of interest to try the effect of alcohol both because of previous studies and because it is itself in many cases a product of respiration. When we introduce a sufficient amount of ethyl alcohol (5 cc. of 96%) to saturate the gas and liquid in the apparatus, we obtain the curves shown in text figure 4. The rate rises rapidly, reaches a maximum, and then falls off in a regular fashion. Here also we find a striking agreement between the curves under aërobic and anaërobic conditions.

The evidence obtained by means of these experiments indicates that

<sup>6</sup> Smith, E. P. Jour. Gen. Physiol. 4: 157. 1921.

aërobic and anaërobic production of carbon dioxid are similar in nature or depend for their progress on a similar master reaction (*i.e.*, the slowest reaction in any consecutive series, which determines the time curves of the final product). Similar results were obtained by Lyon in the case of phosphates. Further experiments must be made before any general conclusion can be reached.

#### SUMMARY

The effects of ether, benzene, and alcohol on the aërobic and anaërobic production of carbon dioxid by wheat are closely similar. This would seem to indicate that the fundamental processes or the master reactions on which they depend are similar.

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ON THE STATUS OF THE GENUS CHAENOCEPHALUS,  
WITH A REVIEW OF THE SECTION LIPACTINIA  
OF VERBESINA

S. F. BLAKE

(Received for publication January 19, 1925)

The genus *Chaenocephalus* of the Heliantheae-Verbesininae, described by Grisebach<sup>1</sup> in 1861 and retained with slightly varying limits by all subsequent authors, is clearly inseparable from the section *Lipactinia* of *Verbesina* established by Robinson and Greenman<sup>2</sup> in 1899. As originally published, the genus was a Jamaican monotype (*C. petrobioides* Griseb.), placed by its author between *Verbesina* and *Salmea*, and compared only with *Salmea*. In the "Genera Plantarum" of Bentham and Hooker it was placed between *Hymenostephium* and *Garcilassa* and in the key to genera was, with *Garcilassa*, separated from *Verbesina*, *Salmea*, *Hymenostephium*, and other genera by its few ("infra 10")-flowered heads. The generic description of Bentham gave the number of flowers in the head as 6-10, but they were described by Grisebach as 12-16, and in heads of *C. petrobioides* dissected by the writer they number 11-26.

In 1879 Grisebach<sup>3</sup> added three Argentinian species to the genus, gave an emended diagnosis, and discussed its relationships as follows:

Genus, novis speciebus auctum, habitu *Petrobio*, generi *Heleniensi* accedens, *Flourensiae* [cf. 478] proximum idemque cum *Verbesina* connectens, distinctum capitulo discoideo, floribus paucioribus et involucrio sensim in paleas transeunte: *Flourensiae* campestri Gr. enim structura florum disci, antherarum stylique eadem et numerus florum parum ultra viginti auctus.

The likeness to *Flourensia* was rather exaggerated by Grisebach, and later writers have not considered the two genera very closely related, the more or less broadly winged achene of *Chaenocephalus*, reinforced by various habitat and technical distinctions, always serving to separate it from *Flourensia*.

Baillon,<sup>4</sup> in 1882, while including in *Verbesina* such widely divergent genera as *Aspilia*, *Blainvillea*, *Helianthella*, and *Wedelia*, retained *Chaenocephalus* as doubtfully distinct, although comparison of his diagnoses of the two genera shows no really differential characters. The heads are described, through a slip of the pen, as 1-16-flowered.

<sup>1</sup> Fl. Brit. W. Ind. 374. 1861.

<sup>2</sup> Proc. Amer. Acad. 34: 563. 1899.

<sup>3</sup> Abh. Ges. Wiss. Göttingen 24: 195-196. 1879.

<sup>4</sup> Hist. Pl. 8: 204. 1882.

O. Hoffmann<sup>5</sup> followed Bentham and Hooker precisely in his arrangement of the genus and in the heading in his generic key ("Kf. homogam, mit weniger als 10 Bl."). In the description of the genus, however, the heads were said to be 6-12-(rarely 16-) flowered. *Verbesina arborea* H. B. K., which has 10-12-flowered heads, was transferred to *Chaenocephalus*, a disposition which has not been accepted by later authors. The leaves of *Chaenocephalus* were described as alternate, but are in fact more often opposite. Since Hoffmann's time there has been no careful consideration of the generic status of *Chaenocephalus*. Urban, who apparently considered all the South American species previously referred to the genus to belong elsewhere, has published three new species from Jamaica and given a key<sup>6</sup> to the four species recognized by him, and another Jamaican species has since been described by Britton. Two South American species described by Hieronymus (1900-1905) have been referred by the writer<sup>7</sup> to the new genus *Monopholis*.

The supposedly distinctive characters of *Chaenocephalus* as contrasted with *Verbesina* that have been brought forward by different authors may be summarized as follows:

Grisebach, 1861. Involucre 1-seriate (2-several-seriate in *Verbesina*); flowers divergent, the corollas curved (straight in *Verbesina*); style branches hispidulous above, with a bluntish point (conical-appendaged in *Verbesina*).

Bentham and Hooker, 1873. Heads 6-10-flowered (many-flowered in *Verbesina*); involucre bracts few, very unequal, passing gradually into the pales (in *Verbesina* few-seriate); anthers auriculate-sagittate at base (obtusely or entire in *Verbesina*).

Grisebach, 1879 (here contrasted with *Flourensia*). Heads discoid, 6-16-flowered (about 20- to many-flowered and radiate in *Flourensia*); involucre 1-2-seriate, grading into the pales (bracts distinct from the pales in *Flourensia*).

Baillon, 1882. No really contrasting distinctive characters.

Hoffmann, 1890. Heads 6-16-flowered (20- or more-flowered in *Verbesina*); fruit margined or narrowly winged (winged, with one exception, in *Verbesina*).

Comparison of the species referred to *Chaenocephalus* with those of the section *Lipactinia* of *Verbesina* shows that none of these differences are sufficiently constant or important to justify the continued separation of *Chaenocephalus*. The style branches in *C. petrobioides*, the type species, are quite as long-appendaged as in any *Verbesina* examined; the anther bases vary somewhat in each group and offer no significant distinction. The heads in *Chaenocephalus* vary (in the Jamaican species) from 6- to 26-flowered, and (in the type species) from 11- to 26-flowered, while among

<sup>5</sup> Engler and Prantl, "Die natürlichen Pflanzenfamilien" 4<sup>5</sup>: 239. 1890.

<sup>6</sup> Symb. Antill. 5: 527. 1908.

<sup>7</sup> Bot. Gaz. 74: 416-420. Pl. 19. 1922.

Verbesinas there are a number of species with less than 20-flowered heads, such as *Verbesina oligantha* Robinson (7), *V. pauciflora* Hemsl. (9), and *V. laevifolia* Blake (8-10). The type species of *Chaenocephalus*, *C. petroboides*, is remarkable for the divergence of the pales and their subtended flowers even before anthesis, and for its divergent or reflexed involucre of 5 linear herbaceous phyllaries sharply distinguished from the blunt whitish pales. In the other Jamaican species the pales are no more divergent than they often are in *Verbesina*. In the other Jamaican species, except *C. rupestris*, the phyllaries are usually about 5, nearly or quite in a single series, and rather readily distinguished from the pales by their herbaceous or subherbaceous tips; in *C. rupestris* they are somewhat more numerous and decidedly unequal, passing gradually into the pales. It is not in *Chaenocephalus* but in many of the *Verbesinas* of the section *Lipactinia* that the greatest difficulty comes in drawing a line between the proper phyllaries and the pales, and the only distinction is often one of position. In the descriptions in this paper and the preceding one,<sup>8</sup> the phyllaries have been considered to include not only the outer empty bracts but also those subtending the rays when these are present, the bracts interior to the rays being definitely pales.

Finally, the alleged difference in the winging of the achenes mentioned by Hoffmann can be dismissed at once. The achenes of all the Jamaican species of *Chaenocephalus* are very definitely winged and differ in no way from those of *Verbesina*.

Once received into the genus *Verbesina*, the species of *Chaenocephalus* do not constitute even a separate group, but fall very naturally into the section *Lipactinia* of Robinson and Greenman. In the revision published in 1899 by these authors, 10 species of this section were recognized; with the species since described, and the addition of *Chaenocephalus*, the number is now 36. It has gradually become apparent that the absence of rays, the principal character, in connection with the small heads, on which the section was based, is not absolutely distinctive of the group, since some of the species related to *V. arborea*, and even that species itself according to Hieronymus, occasionally or always have a few small rays. As here taken, the section *Lipactinia* includes all the discoid species with small or medium-sized heads, as well as a smaller number of small-rayed plants that are clearly very closely allied to some of the typical discoid species, particularly to *V. arborea*.

The preparation of this revision has been greatly aided by the loan of material from the Gray Herbarium, the herbarium of the New York Botanical Garden, and the herbarium of the Field Museum, for which the writer's thanks are due to the authorities of these institutions. In the citation of herbaria, F = herb. Field Museum; G = Gray Herbarium; N = U. S. National Herbarium; Y = herb. New York Botanical Garden. Informa-

<sup>8</sup> Blake. New South American *Verbesinas*. Bull. Torrey Bot. Club 51: 421-436. 1924.

tion regarding the types of three species in the Kew Herbarium, furnished by Mr. John Hutchinson, has supplemented Baker's descriptions and enabled me to key these species properly. In the enumeration the species have been arranged in what appears to be a somewhat natural sequence, which it has not been possible to follow very closely in the key.

#### KEY TO THE SPECIES OF VERBESINA SECTION LIPACTINIA

##### A. Jamaican species; leaves usually cuneate or wedge-obovate and coarsely toothed; corollas white or whitish

- Leaves glabrous on both sides, coriaceous, sessile, long-decurrent on the stem; pales strongly divergent even before anthesis.....1. *V. petrobioides*.  
 Leaves pubescent on both sides (or nearly glabrous in *V. nervosa* and *V. propinqua*), rarely coriaceous, very rarely short-decurrent; pales not strongly divergent at anthesis.  
 Leaves borne on short but distinct naked petioles; corolla teeth from  $\frac{3}{5}$  as long to as long as the throat.  
 Heads 15-20-flowered; leaves smooth on both sides, glabrous except for a slight puberulence along the veins beneath.....2. *V. nervosa*.  
 Heads 6-10-flowered; leaves very rough above, conspicuously pubescent on both sides.....3. *V. rupestris*.  
 Leaves sessile; corolla teeth less than half as long as throat.  
 Leaves very rough on both sides from the persistent, whitish, tuberculate hair-bases, coarsely dentate-lobate or sometimes merely serrate.....4. *V. aspera*.  
 Leaves obscurely tuberculate-hispidulous or nearly glabrous on both sides, sharply toothed but not lobed.....5. *V. propinqua*.

##### B. Mexican and Central American species; heads 7-9-flowered

- Leaves elliptic-oblong, essentially glabrous, alternate; Costa Rica....6. *V. trichantha*.  
 Leaves lance-oblong to ovate, pubescent, opposite; Mexico.  
 Leaf blades ovate, green and harshly pubescent beneath; stem scabrid-puberulous.....25. *V. oligantha*.  
 Leaf blades lance-oblong, cinereously hirsute-subtomentose beneath; stem glabrous.....26. *V. pauciflora*.

##### C. South American species

- Leaves pinnately about 9-lobed, very large, alternate; corollas white (color not known in *V. bipinnatifida*).  
 Leaves griseous-pubescent beneath, the lower segments pinnatifid; achenes distinctly winged (wings 1 mm. wide above); Brazil.....34. *V. bipinnatifida*.  
 Leaves densely and rather softly griseous-pilosulous beneath, the segments merely serrate; axis of panicle sordid-pilosulous; heads 20-22-flowered, the disk 6-10 mm. high; achenes very narrowly winged; Colombia.....35. *V. crassicaulis*.  
 Leaves densely and somewhat harshly subsericeous-hispidulous beneath with ochroleucous hairs, the lower segments sometimes pinnatifid-lobed; axis of panicle glabrous; head 13-15-flowered, the disk 5 mm. high; achenes obsoletely winged; Ecuador.....36. *V. minuticeps*.



Leaves unlobed, or in a few species 3-5-lobed.

Heads 40-90-flowered; leaves alternate.

Leaf blades 2.8-6.5 cm. long; Colombia.....30. *V. baccharidea*.

Leaf blades much larger; British Guiana, Brazil.

Leaf blades 10-15 cm. long, 3-5 cm. wide.

Heads 80-90-flowered; Brazil.....31. *V. claussemi*.

Heads about 40-flowered; British Guiana.....32. *V. schomburgkii*.

Leaf blades 19-30 cm. long, 6-9 cm. wide; Brazil.....33. *V. floribunda*.

Heads 4-24-flowered; leaves alternate or opposite.

Heads 13-24-flowered.

Stem conspicuously winged throughout.

Leaves coarsely 3-5-lobed, merely hispidulous beneath; heads discoid; Argentina.....11. *V. lilloi*.

Leaves unlobed, subtomentose beneath; heads radiate; Ecuador.....12. *V. brachypoda*.

Stem wingless, or the petiole bases rarely short-decurrent.

Petioles auriculate, usually winged throughout; phyllaries oblong to oval or oval-ovate, rather densely puberulous; Colombia, Peru.....22. *V. callacatensis*.

Petioles not auriculate, or else (*V. lloensis*) the phyllaries linear or linear-lanceolate, sparsely pubescent, acute or acuminate.

Leaves sessile, pubescent beneath only on the veins; disk about 1 cm. thick; Bolivia.....21. *V. flavovirens*.

Leaves petioled (sessile in *V. guianensis*?), pubescent on the surface as well as on the veins beneath; disk usually smaller.

Leaves opposite essentially throughout.

Outermost phyllaries linear-oblong or spatulate-oblong, 1 mm. wide or less, the inner 1-1.3 mm. wide; teeth of leaves slender, acutish, 1 mm. high; leaves densely hirsutulous-pilosulous beneath....17. *V. adenobasis*.

Outermost phyllaries oblong, 1.2-1.5 mm. wide, the inner 2 mm. wide; teeth of leaves depressed, apiculate, 0.5 mm. high; leaves densely subtomentose-pilosulous beneath.....18. *V. latisquama*.

Leaves chiefly or entirely alternate.

Phyllaries linear or linear-lanceolate, acute or acuminate; Ecuador.....19. *V. lloensis*.

Phyllaries oblong to oblong-oblancheolate or obovate, obtuse.

Heads radiate, on pedicels usually 1-2 cm. long; Peru.....20. *V. grandifolia*.

Heads discoid.

Heads 5-15-flowered; Colombia.

Heads mostly sessile, in small glomerules; phyllaries with distinctly subherbaceous tips.....14. *V. crassiramea*.

Heads mostly pedicellate; phyllaries without distinctly subherbaceous tips.....16. *V. pennellii*.

Heads about 20-flowered; Brazil,  
Guiana.

Leaves 10–15 cm. long, sparsely  
pubescent or glabrate be-  
neath; heads short-pedicelated...28. *V. guianensis*.

Leaves 23–30 cm. long, persist-  
ently whitish-tomentose be-  
neath; heads slender-pedi-  
celated.....29. *V. nicotianaefolia*.

Heads 4–12-flowered.

Leaves strictly glabrous on both sides.

Leaves opposite, oval to oblong-elliptic, sessile; Peru.....23. *V. laevis*.

Leaves alternate, wedge-obovate or elliptic-obovate,  
short-petioled; Venezuela.....27. *V. laevifolia*.

Leaves pubescent on both sides.

Leaves merely puberulous or sparsely tuberculate-  
hispidulous beneath.

Leaves rather sparsely tuberculate-hispidulous be-  
neath; pales cymbiform, nearly glabrous; Peru....24. *V. jelskii*.

Leaves puberulous beneath; pales narrow, not  
cymbiform.

Heads 4–5-flowered; Ecuador.....7. *V. pentantha*.

Heads 8–11-flowered; Argentina.

Heads 9–11-flowered; leaves mostly oppo-  
site, subentire (the lower sometimes 3-  
lobed); involucre 2–3-seriate.....8. *V. suncho*.

Heads about 8-flowered; leaves alternate,  
unequally and doubly serrate above; in-  
volucre several-seriate.....9. *V. octantha*.

Leaves tomentose or subtomtose beneath.

Petioles auriculate, usually winged throughout;  
phyllaries oblong to oval or oval-ovate, obtuse to  
short-pointed; Colombia, Peru.....22. *V. callacatensis*.

Petioles not auriculate, or else (*V. lloensis*) the  
phyllaries very narrow, acute to acuminate.

Phyllaries linear or linear-lanceolate, acuminate  
or acute; heads 12–18 (or more?)-flowered;  
rays often present; Ecuador.....19. *V. lloensis*.

Phyllaries oblong or ovate to obovate, obtuse,  
rounded, or sometimes short-pointed, or  
if narrower and subacuminate (*V. allo-  
phylla*) then heads discoid, narrow, 11–  
12-flowered, in dense, fastigate, cymose  
panicles.

Leaves usually opposite and (at least the  
lower) 3–5-lobed; heads discoid, slender,  
pedicellate, in dense, flattish, fastigate,  
cymose panicles; phyllaries mostly linear  
or lanceolate, densely and finely puber-  
ulous; Argentina, Bolivia.....10. *V. allophylla*.

Leaves alternate (opposite in *V. latisquama*),  
very rarely lobed; heads in usually  
rounded panicles; phyllaries mostly

- oblong or obovate-oblong and obtuse  
or rounded; Colombia to Bolivia.  
Leaves opposite; heads radiate; Ecuador.....18. *V. latisquama*.  
Leaves alternate; heads nearly always  
discoid.  
Phyllaries with obscurely if at all  
herbaceous tips; Colombia.....16. *V. pennellii*.  
Phyllaries (at least the outer) with  
definitely herbaceous or sub-  
herbaceous tips.  
Phyllaries and upper part of pales  
densely hirsutulous-pilosulous;  
Ecuador.....15. *V. arborea*.  
Phyllaries ciliolate and sparsely  
puberulous; pales rather  
sparsely pubescent above.  
Heads 5--6-flowered, usually  
short-pedicel; leaves ob-  
long; Bolivia.....13. *V. cumingii*.  
Heads 5--14-flowered, most-  
ly sessile in small glomer-  
ules; leaves ovate; Colom-  
bia.....14. *V. crassiramea*.

1. *Verbesina petrobioides* (Griseb.) Blake.

*Chaenocephalus petrobioides* Griseb. Fl. Brit. W. Ind. 374. 1861.

JAMAICA: Harris 8806 (Y), 12502 (G, N, Y), 12778 (N, Y).

Distinguished from all the other Jamaican species by its long-decurrent leaf bases, large and perfectly glabrous leaves, and pales strongly divergent even before anthesis.

2. *Verbesina nervosa* Blake, nom. nov.

*Chaenocephalus venosus* Urban. Symb. Antill. 5: 525. 1908. Not

*Verbesina venosa* Greene. 1882.

JAMAICA: Harris 9109 (TYPE COLLECTION; Y).

Distinguished among the Jamaican species by its smooth, petioled leaves.

3. *Verbesina rupestris* (Urban) Blake.

*Chaenocephalus rupestris* Urban. Symb. Antill. 3: 412. 1903.

JAMAICA: Harris 7810 (N, Y), 7811 (N, Y), 7813 (N, Y), 9125 (N, Y).

Separated from the other Jamaican species by its rough, petioled leaves. Harris 7810 differs from all the other specimens examined in having the petioles auriculate at base, with the auricles sometimes short-decurrent.

4. *Verbesina aspera* Blake, nom. nov.

*Chaenocephalus lobatus* Urban. Symb. Antill. 5: 526. 1908. Not  
*Verbesina lobata* Gaud. 1826.

*Chaenocephalus lobatus* var. *brachyphyllus* Urban. Symb. Antill. 5: 527.  
1908.

JAMAICA: *Harris* 9510 (N), 10005 (TYPE COLLECTION of var. *brachyphyllus*; N, Y), 10058 (N), 11652 (G, N, Y).

Its very rough-tuberculate, sessile, coarsely toothed or lobate leaves separate this from all the other Jamaican species. Urban's var. *brachyphyllus*, the type collection of which has been examined, is not worthy of recognition by name.

5. *Verbesina propinqua* (Britton) Blake.

*Chaenocephalus propinquus* Britton. Bull. Torrey Bot. Club 37: 360. 1910.

JAMAICA: *Britton* 1144 (TYPE; Y), *Harris* 9672 (N).

Characterized among Jamaican species by its sessile, nearly smooth leaves. A single head of the type dissected was 18-flowered.

6. *Verbesina trichantha* (Kuntze) Blake.

*Chaenocephalus petrobiodes* var. *trichanthus* Kuntze. Rev. Gen. Pl. 1: 327. 1891.

Stem (or branch) stout, sordid-hirsutulous, glabrescent; leaves alternate, the lower elliptic-oblong, about 15 cm. long and 4 cm. wide, apparently acute or acuminate, sessile (?), callous-serrulate (teeth 0.5–0.8 mm. high, 3–7 mm. apart) submembranous, glabrous or essentially so on both sides, finely reticulate, the upper elliptic, subentire, sessile, about 5 cm. long, 1.5 cm. wide; panicle densely sordid-hirsutulous with mostly spreading hairs, about 10 cm. wide, many-headed, the heads discoid, about 7-flowered, mostly sessile and glomerulate, about 9 mm. high in young fruit; involucre about 1-seriate, about 4.5 mm. high, the phyllaries very unequal, oval to linear-oblong, obtuse or the inner acutish, thick-subherbaceous, hispidulous-ciliolate, sparsely hispidulous or subglabrous on back; corollas densely hirsutulous on tube and lower throat, papillose on inner margin of teeth, 5.3 mm. long (tube 1.8 mm., throat slightly widened above, 1.5 mm., teeth nearly linear, acute or acuminate, 2 mm. long); pales obtuse or acutish, hispidulous-ciliolate above, sparsely hispidulous or glabrous on back, about 7 mm. long; achenes cuneate-obovate, flat, 1-ridged on the sides, thickened on margin, not winged, hirsutulous, 5 mm. long, 2.3 mm. wide; awns 2, upwardly hirsutulous, equal or unequal, 2.5–3.2 mm. long; style branches with acuminate, hispidulous appendages.

COSTA RICA: Without definite locality, 1874, *Kuntze* (TYPE COLLECTION; N, Y).

The extremely fragmentary material of this species does not suffice for satisfactory description, although it leaves no doubt that the species is distinct. Its nearest alliance is probably with the Jamaican species, but it differs from all of them in having the corolla teeth longer than the throat, as well as in many other features.

7. *Verbesina pentantha* Blake. Bull. Torrey Bot. Club 51: 435. 1924.

ECUADOR: Between Santa Rosa and La Chorita, Prov. Oro, *Hitchcock* 21144 (TYPE; N).

Distinguished by its 4–5-flowered heads and merely puberulent stem and leaves.

8. *Verbesina suncho* (Griseb.) Blake.

*Chaenocephalus suncho* Griseb. Abh. Ges. Wiss. Göttingen 24: 195. 1879.

ARGENTINA: Tabacal near Oran, *Lorentz & Hieronymus* 528 (TYPE COLLECTION; Y, sketch and fragm. G.).

Distinguished by its puberulous stem, mostly opposite, oblong, short-petioled, subentire leaves (the lower sometimes 3-lobed, according to Grisebach), these puberulous beneath, finely puberulent involucre, and small, discoid 9- -11-flowered heads in close, flattish-topped cymes.

9. *Verbesina octantha* Blake, nom. nov.

*Chaenocephalus macrophyllus* Griseb. Abh. Ges. Wiss. Göttingen 24: 196. 1879. Not *Verbesina macrophylla* (Cass.) Blake. Bull. Torrey Bot. Club 51: 430. 1924.

Based on specimens from Tucumán. Characterized by its puberulent branches, alternate, large (20 cm. long), doubly serrate, petioled leaves which are puberulent beneath, and about 8-flowered heads. Lillo (see citation under no. 11) states that wings are present on the young shoots of this species, disappearing by flowering time. No specimens have been examined.

10. *Verbesina allophylla* Blake, nom. nov.

*Chaenocephalus heterophyllus* Griseb. Abh. Ges. Wiss. Göttingen 24: 196. 1879. Not *Verbesina heterophylla* (Chapm.) A. Gray. 1883.

BOLIVIA: Machichoirisa, alt. 1065 m., 1902, *Williams* 1592 (Y); in cultivated ground, Huachi, head of Beni River, alt. 915 m., 1921, *Rusby* (*Mulford Biol. Expl.* 458; N, Y).

ARGENTINA: Prov. Jujuy, *R. E. Fries* 284 (N); Tucumán, *Kuntze* (N, Y); near Oran, *Lorentz & Hieronymus* 343 (sketch and fragm. of TYPE; G).

Recorded also by Grisebach from Tarija, Bolivia. Distinguished by its mostly opposite, usually 3-lobed, broadly ovate leaves, subtomentose beneath, and small, discoid 11- -12-flowered heads in dense, fastigate, cymose panicles. In Kuntze's specimens narrow wings are present on the stem decurrent from the bases of some of the petioles.

11. *Verbesina lilloi* Blake, nom. nov.

*Chaenocephalus alatus* Lillo, "Reseña Fitogeográfica de la Provincia de Tucumán," in Primera Reunión Nacional de la Sociedad Argentina de Ciencias Naturales: Tucumán, 1916: 226. 1919. Not *Verbesina alata* L. 1753.

"Shrubby, 1 m. high;" stem stout, puberulous, winged to the inflorescence (wings 2 mm. wide); leaves alternate or sometimes opposite; naked portion of petiole 2-10 mm. long; blades broadly ovate in outline, 9-19 cm. long, 11-16 cm. wide or more, deeply 3- -5-lobed (the lobes acuminate, serrate or the lower with lateral lobes), acuminate, decurrent on petiole nearly to its base, harshly tuberculate-hispidulous above, harshly, finely,

and rather sparsely hispidulous chiefly on the veins and veinlets beneath, triplinerved; heads numerous in cymose panicles at apex of stem and branches, discoid, 18-23-flowered, yellow; disk 8 (young fruit) to 13 mm. high, 5 to (fruit) 12 mm. thick; involucre about 2-seriate, graduate, 5-6.5 mm. high, the phyllaries linear-oblong or lance-oblong, obtusish to acute, blackish-green and puberulous below, with subglabrous, subherbaceous, rather loose tips; corollas hirsute-pilose on tube and base of throat, sparsely hirsutulous on teeth, 6 mm. long (tube 1.8 mm., teeth 1 mm.); pales acute or acuminate, sparsely puberulous, about 8 mm. long; achenes oblong-obovate, flat, 1-ridged on each side, hirsutulous, 2-winged, 6.5 mm. long, 2.8-3.5 mm. wide (including wings, these 0.5-1 mm. wide, sparsely hispidulous-ciliolate); awns 2, upwardly hispidulous, unequal or equal, 3-5 mm. long.

ARGENTINA: Common, El Candado, Dept. Andalgalá, Prov. Catamarca, 1916 and 1917, *Jørgensen* 1287 (G, N).

The above description, drawn up from specimens distributed as *C. alatus* Lillo, has been supplied because of the insufficiency of the original diagnosis, which merely distinguished the species from *C. macrophyllus* Griseb. by the permanently winged stems. Lillo states that the plant is not rare in alder woods in the Province of Tucumán.

12. VERBESINA BRACHYPODA Blake. Bull. Torrey Bot. Club 51: 434. 1924.

ECUADOR: Cuenca, *Holway & Holway* (TYPE COLLECTION; G, Y, fotogr. and fragm. N.).

Distinguished by its winged stem, its alternate, ovate, unlobed leaves submentose beneath and on short, winged petioles, its small, about 15-flowered, radiate heads, and its short, slightly pubescent involucre of obtuse or rounded, blackish-green phyllaries. The species serves to connect the *V. arborea* group and the *V. allophylla* group.

13. *Verbesina cumingii* Schultz Bip., sp. nov.

*Verbesina cumingii* Schultz Bip. Linnaea 34: 528. 1865-66, and Bull. Soc. Bot. France 12: 79. 1865, nomen nudum.

*Chaenocephalus cumingii* Griseb. Abh. Ges. Wiss. Göttingen 24: 196. 1879, hyponym.

Stem cinereously lanate-tomentose; leaves alternate; petioles naked, stout, lanate-tomentose, 1-2 cm. long; blades oblong or ovate-oblong, up to 24 cm. long, 7.5 cm. wide, acuminate, acute at base, serrate or the upper subentire, papery, tuberculate-hispidulous and scabrid above, beneath densely pilose-tomentose with cinereous or ochroleucous hairs, feather-veined, reticulate beneath; heads discoid, 5-6-flowered, very numerous in a large, dense terminal cymose panicle; pedicels mostly 2-5 mm. long, rarely obsolete; disk narrow, in anthesis 8-10 mm. high; involucre about 3-seriate, graduate, about 4 mm. high, the phyllaries few, oblong or oval-oblong, obtuse to acutish, blackish-green with pale, subscarios margin and apex, ciliate and rather sparsely sordid-pilosulous; corollas yellow, pilose on tube and lower part of throat, glabrous above, 5.5 mm. long; pales similar to the phyllaries but longer, acutish; achenes (immature) cuneate, glabrous, wingless; awns 2, unequal, about 3 mm. long.

ECUADOR: In woods, near Soratá, alt. 2800–3100 m., July–August 1858–1859, *Mandon* 56 (TYPE in Gray Herb.; duplicate in herb. N. Y. Bot. Gard.; fotogr. and fragm., U. S. Nat. Herb.).

The name *Verbesina cumingii* was first published in Schultz Bipontinus' list of Mandon's Compositae (no. 56), where *Cuming* 108 or 1108 (108 in Linnaea list, 1108 in list in Bull. Soc. Bot. France) was also mentioned. Since Cuming's plant is not accessible in this country, and since Mandon's plant was equated with it by Schultz himself, it seems necessary for the purposes of this revision to take the Mandon collection as the type of the specific name. *Verbesina cumingii* was referred to *V. arborea* H. B. K. by Robinson and Greenman, but in that species the heads are 10–12-flowered and usually sessile, the pubescence of stem and leaves is much darker and more sordid, and the phyllaries and pales are more densely pubescent. Specimens collected by O. E. White (Mulford Biol. Expl. 155; N, Y), at Pongo, Bolivia, alt. ca. 3500 m., 1921, are referred to *V. cumingii* with little hesitation. They differ chiefly in the duller and shorter pubescence of the stem and lower leaf surface and the pale phyllaries.

14. VERBESINA CRASSIRAMEA Blake. Contr. U. S. Nat. Herb. 22: 640. 1924.

COLOMBIA: Vicinity of Bogotá, *Apollinaire & Arthur* 13 (TYPE; N), *Ariste-Joseph* A 32 (N), A 385 (N), A 428 (N), A 523 (N), A 527 (N), *Holton* 371 (Y); San Cristóbal, Dept. Cundinamarca, *Pennell* 2280 (Y).

The further collections of this species examined since it was described show heads ranging from 5- to 14-flowered, but only rarely less than 8-flowered. The achene wings, scarcely developed in the original specimens, are sometimes 1.5 mm. wide. The species is distinguished by its sordidly tomentose or lanate-tomentose stems and lower leaf surface, its large, alternate, subentire or slightly serrulate leaves on long naked petioles, its mostly sessile and glomerulate 5–14-flowered heads, and its rather sparsely pubescent phyllaries, the outer usually somewhat widened above and with definitely subherbaceous tips.

15. VERBESINA ARBOREA H. B. K. Nov. Gen. et Sp. 4: 202. 1820.

*Chaenocephalus arboreus* O. Hoffm. in Engler and Prantl, Nat. Pflanzenfam. 4<sup>5</sup>: 239. 1890.

ECUADOR: A shrub, Malchinguí to Pomasqui, Prov. Pichincha, alt. 3000–3600 m., August 13, 1923, *Hitchcock* 20845 (N), 20869 (N).

Originally described from specimens with unopened flowers collected in "frigidis Regni Quitensis, praesertim in declivitate montis Pichincae, alt. 1700 hex." Hitchcock's specimens, which are in mature fruit, agree so well with the original description that I have no hesitation in referring them to this species. The species is close to *V. crassiramea*, differing chiefly in its more densely pubescent involucre. The achene wings are 1.2–1.8 mm. wide. According to Hieronymus (Bot. Jahrb. Engler 29: 45. 1900), the

heads of this species sometimes have a few whitish rays (*Sodi* 37/2, from Lloa Valley, on the western slopes of Pichincha).

16. VERBESINA PENNELLII Blake. Contr. U. S. Nat. Herb. 22: 640. 1924.

COLOMBIA: Dept. Huila, *Pennell* 992 (TYPE COLLECTION; N, Y); Dept. El Valle, *Pennell & Killip* 6081 (N), *Pennell, Killip, & Hazen* 8515 (N); Dept. Cundinamarca, *Ariste-Joseph* 1009 (N).

In the original description the corollas were said to be whitish. In no. 6081 they are described as pale yellow, and in 8515 as yellowish-white. In the latter collection the heads are 15-flowered. A large leaf of no. 6081 has a blade 19 by 10 cm., and is sinuately 2-lobed on each side near the middle. Both this and *V. crassiramea* are perhaps too close to *V. arborea*.

17. VERBESINA ADENOBASIS Blake. Bot. Gaz. 74: 425. 1922.

ECUADOR: Cuenca, *Holway & Holway* 991 (TYPE; N).

Distinguished by its sordid-pilulous branches, opposite, broadly ovate leaves, about 14-flowered heads with few small rays, and linear-oblong or spatulate-oblong, sparsely puberulous phyllaries.

18. VERBESINA LATISQUAMA Blake. Bot. Gaz. 74: 426. 1922.

ECUADOR: Cuenca, *Holway & Holway* 994 A (TYPE; N).

Closely allied to *V. adenobasis*, but differing in the characters given in the key.

19. VERBESINA LLOENSIS Hieron. Bot. Jahrb. Engler 29: 45. 1900.

ECUADOR: Lloa, *Sodi* 37/4 (TYPE COLLECTION; fragm. N).

Distinguished by its tomentose branches and lower leaf surface, sometimes auriculate petioles, large, chiefly alternate, oblong leaves, rather long pedicels (1-1.5 cm.), about 18-flowered heads, and narrow acute or acuminate phyllaries. The var. *lobata* of Hieronymus (op. cit. 46), with broader lobate leaves, seems doubtfully worthy of recognition by name.

20. VERBESINA GRANDIFOLIA Blake. Bull. Torrey Bot. Club 51: 432. 1924.

PERU: Mito, *Macbride & Featherstone* 1500 (TYPE COLLECTION; F, N).

Characterized by its tomentose stem and lower leaf surface, alternate, very large, unlobed leaves, rather long pedicels (mostly 1-2 cm. long), about 18-flowered heads, and oblong or obovate-oblong, obtuse or rounded, subherbaceous-tipped phyllaries.

21. VERBESINA FLAVOVIRENS R. E. Fries. Arkiv Bot. 5<sup>13</sup>: 19. Pl. 2, figs. 1-3. 1906.

Based on *Fries* 1292, from Pinos, near Tarija, Bolivia. Characterized by its low stature (1.5 m.), its alternate, opposite, or ternate, medium-sized, lanceolate or rhombic-elliptic, sessile leaves, which are scabrous above and long-pilose only on the nerves beneath, and its medium-sized, about 16-flowered, rather long-pedicelled heads. No specimens have been examined.



22. VERBESINA CALLACATENSIS Hieron. Bot. Jahrb. Engler 36: 492. 1905.  
COLOMBIA: *Ariste-Joseph* (N); Dept. El Cauca, *Killip* 6817 (N); Popayan, *Lehmann* (Bentham Trustees' no. 576; G, Y, photogr. and fragm. N).

PERU: Callacate, *Jelski* 649 (TYPE COLLECTION; fragm. N).

An exceedingly variable species, if my reference of the Colombian material to it is correct. In these specimens the leaves are alternate or very rarely opposite; the petioles are either winged to base, and then auriculate and sometimes decurrent as broad wings, or naked above and auriculate at base; the heads are sometimes discoid and 9-17-flowered, sometimes radiate and 21-flowered (rays 3, very small, disk flowers 18). The species is characterized by its dense, sordid or griseous, tomentose pubescence, its auriculate, usually winged petioles, its large, nearly always alternate leaves, its obtuse, densely hirsutulous or puberulous phyllaries, and its 9-21-flowered heads.

23. VERBESINA LAEVIS Blake. Contr. U. S. Nat. Herb. 22: 639. 1924.

PERU: Prov. Chachapoyas, *Mathews* (TYPE COLLECTION; G, Y, photogr. and fragm. N).

Distinguished by its glabrous and glaucous stem, small, opposite, sessile, subentire, coriaceous, glabrous and glaucous leaves, and 10-flowered discoid heads.

24. VERBESINA JELSKII Hieron. Bot. Jahrb. Engler 36: 493. 1905.

PERU: Near Tambillo, *Jelski* 691 (TYPE COLLECTION; fragm. N), 741 (fragm. N).

Distinguished by its puberulous, glabrate stem, its mostly opposite, petioled, elliptic-oblong, essentially entire leaves, rather sparsely tuberculate-hispidulous on both faces, its discoid 6-8-flowered heads, and its firm, cymbiform, nearly glabrous pales. Hieronymus described the heads as constantly 6-flowered, but a head of *Jelski* 741 dissected by the writer was 8-flowered.

25. VERBESINA OLIGANTHA Robinson. Proc. Amer. Acad. 47: 214. 1911.

MEXICO: Jimalcota, Michoacán or Guerrero, *Langlassé* 644 (TYPE COLLECTION; G, N).

Distinguished by its wingless, scabrous-puberulent stem, opposite, ovate or elliptic-ovate, serrate, rather large, harsh leaves, and discoid 7-flowered heads.

26. VERBESINA PAUCIFLORA Hemsl. Biol. Centr. Amer. Bot. 2: 189. 1881.

*Verbesina cymosa* A. Gray. Proc. Amer. Acad. 21: 390. 1886.

CHIHUAHUA: Near Batopilas, 1885, *Palmer* 135 (TYPE COLLECTION of *V. cymosa*; N).

Characterized by its glabrous stem, opposite, serrate, lance-oblong

leaves, harsh above and cinereous-subtomentose beneath, and discoid 7-9-flowered heads. Hemsley's type (*Seemann* 1468) came from Cerro de Pinal, Sinaloa.

27. *VERBESINA LAEVIFOLIA* Blake. Contr. U. S. Nat. Herb. 20: 540. Pl. 47. 1924.

VENEZUELA: Silla de Caracas, *Pittier* 8332 (TYPE; N).

Distinguished by its glabrous stem, alternate, crowded, mostly wedge-obovate, subentire, glabrous leaves, and discoid, 8-10-flowered, mostly glomerulate, white heads.

28. *VERBESINA GUIANENSIS* Baker in Mart. Fl. Bras. 6<sup>3</sup>: 211. 1884.

Based on *Schomburgk* 194 and 654 from British Guiana. Characterized by its wingless stem, its alternate, "sessile," oblanceolate-oblong leaves 10-15 cm. long, scabrous above and sparsely pubescent or glabrate beneath, its discoid heads 9-10 mm. thick, and its rather broadly winged achenes. Mr. John Hutchinson, who has examined the types in the Kew Herbarium, informs me that the heads are about 20-flowered and that the pales (described as glabrous by Baker) are loosely pubescent above.

29. *VERBESINA NICOTIANAEOFOLIA* Baker in Mart. Fl. Bras. 6<sup>3</sup>: 212. 1884.

Based on *Pohl* 621, from an unknown locality in Brazil. Characterized by its wingless stem, its alternate, subpetioled, oblong-spatulate leaves 23-30 cm. long, hispidulous above and persistently whitish-tomentose beneath, its discoid heads 6-8 mm. thick, and its narrowly winged achenes. Mr. Hutchinson informs me that the heads are about 20-flowered and the pales loosely pubescent above.

30. *VERBESINA BACCHARIDEA* Blake. Contr. U. S. Nat. Herb. 22: 641. Pl. 60. 1924.

COLOMBIA: Near Bogotá, *Ariste-Joseph* A 245 (TYPE; N), *Rusby & Pennell* 1270 (N), *Pennell* 2012 (Y).

Characterized by its small, alternate, chiefly elliptic, crowded leaves, lanate-pilose involucre, and discoid, about 60-flowered, white heads.

31. *VERBESINA CLAUSSENI* Schultz Bip.; Baker in Mart. Fl. Bras. 6<sup>3</sup>: 212. 1884.

Described by Baker from several collections made in Minas Geraes, Brazil. Characterized by its densely pilose, wingless stem, its alternate, subpetiolate, oblanceolate-oblong leaves 10-12.5 cm. long and densely pubescent beneath, its very many-flowered discoid heads 1-1.2 cm. thick, and its narrowly winged achenes. Mr. Hutchinson writes me that the heads are about 80-90-flowered and that the pales are loosely pubescent above.

32. *VERBESINA SCHOMBURGKII* Schultz Bip. (in *Schomb. Versuch Fauna und Fl. Brit. Guiana* 1078. 1848, nomen); Klatt. *Leopoldina* 20: 94. 1884.

BRITISH GUIANA: Mt. Roraima, *Schomburgk* 993 (TYPE COLLECTION; sketch and fragm., G), 1884, *Jenman* 86 (N).

Distinguished by its wingless stem, its alternate, petioled, elliptic or cuneate-elliptic leaves about 11.5 cm. long, rough above and evenly but not densely hirsutulous beneath, its discoid 40-flowered heads, and its densely pubescent pales.

33. *VERBESINA FLORIBUNDA* Gardn. Lond. Jour. Bot. 7: 407. 1848.

BRAZIL: Prov. Minas Geraes, *Gardner* 4927 (TYPE COLLECTION; N).

Distinguished by its wingless, densely appressed-puberulous stem, its large, alternate, petioled leaves, appressed-puberulous beneath, its discoid, about 45-flowered heads, and its broadly winged achenes.

34. *VERBESINA BIPINNATIFIDA* Baker in Mart. Fl. Bras. 6<sup>3</sup>: 213. 1884.

Based on specimens collected by Martius in the Province of Minas Geraes, Brazil. Distinguished by its large, alternate, pinnately lobed leaves, griseous-pubescent beneath and with pinnatifid lower segments, its small discoid heads, and distinctly winged achenes. Baker mentions a related, undescribed species collected by Spruce (no. 5969) on the Río Chanchan, Peru.

35. *VERBESINA CRASSICAULIS* Blake. Bull. Torrey Bot. Club 51: 430. 1924.

COLOMBIA: La Cumbre, Dept. El Valle, Cordillera Occidental, *Pennell* 5200 (TYPE; N).

Distinguished by its glabrous stem, its alternate, very large, deeply pinnate-lobed leaves, densely griseous-pilosulous beneath and with merely serrate segments, its 20-22-flowered heads, and its very narrowly winged achenes.

36. *VERBESINA MINUTICEPS* Blake. Bull. Torrey Bot. Club 51: 431. 1924.

ECUADOR: Guayaquil, *Hitchcock* 20159 (TYPE; N).

Characterized by its glabrous, wingless stem, its alternate, very large, deeply pinnate-lobed leaves, rather harshly subsericeous-hispidulous beneath and with sometimes pinnatifid lower segments, its about 15-flowered tiny heads, and its obsoletely winged achenes.

#### EXCLUDED SPECIES

*Chaenocephalus jelskii* Hieron. Bot. Jahrb. Engler 36: 494. 1905.

This is *MONOPHOLIS JELSKII* (Hieron.) Blake, Bot. Gaz. 74: 420. 1922.

*Chaenocephalus pallatangensis* Hieron. Bot. Jahrb. Engler 29: 47. 1900.

This is *MONOPHOLIS PALLATANGENSIS* (Hieron.) Blake, Bot. Gaz. 74: 419. 1922.

VERBESINA AURICULATA DC. Prodr. 5: 617. 1836.

Described by De Candolle as having discoid heads, and placed in the section *Lipactinia* by Robinson and Greenman. Fragments from the type (*Andrieux* 302, from Tehuantepec, Oaxaca), subsequently obtained by Dr. Robinson and now in the Gray Herbarium, although very immature serve to show that the heads are provided with small, apparently rather numerous rays (oval, apparently bidentate, about 2.5 mm. long). In the very old type these appear whitish, but may have been pale yellow. The proper place of the species is probably in the section *Saubinetia* next to *V. potosina* Robinson and *V. oreopola* Robins. & Greenm. It is well distinguished by its large, alternate leaves, scabrous above and densely sordid-tomentulose beneath, on winged, auriculate, but not decurrent petioles, its rather large heads (disk about 1.2 cm. thick when young), its short, broadly ovate, sordid-tomentulose phyllaries with indurate base and short obtuse or acute subherbaceous tips, and its narrow, very sparsely pilose pales with stiffly acuminate, almost cuspidate, erect or slightly recurved tips. The only known collection beside the type is one made by Liebmann (no. 211) at "Villa Alta, Mexico," represented in the Gray Herbarium by an excellent sketch from the Klatt herbarium.

*Verbesina grisebachii* Baker in Mart. Fl. Bras. 6<sup>3</sup>: 214. 1884.

This is *ZEXMENIA GRISEBACHII* (Baker) Hassl. Rep. Spec. Nov. Fedde 14: 157. 1915. Hassler states that the heads are radiate.

VERBESINA HOLWAYI Robinson. Proc. Amer. Acad. 51: 539. 1916.

This species, described from over-ripe material, was doubtfully referred to the section *Lipactinia*. A later collection (no. 737 of 1917) in the Gray Herbarium, made by Professor Holway at the type locality, Quezaltenango, Guatemala, has heads with about 8 whitish rays, these about 3 mm. long, and shows that the species belongs in the section *Ochractinia*.

# THE RESPIRATORY ACTIVITY OF VARIOUS PARTS OF THE CRANBERRY PLANT IN RELATION TO FLOODING INJURY

H. F. BERGMAN

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Injury to various parts of the cranberry plant as a result of flooding has been shown (1) to be due to oxygen deprivation. The liability to injury is proportional to the rate of respiration of the various parts. The buds, blossoms, and tips of vines as the most active parts are most seriously injured. In extreme cases all the buds and blossoms may be killed, this resulting in a total loss of the crop. The rate of respiration of buds and young fruits as compared with blossoms and growing tips of the same variety with reference to their liability to flooding injury was not studied. Nor was the possibility of variation in the rate of respiration of corresponding parts in other varieties considered.

The volumetric method employed in the former work measured the amount of carbon dioxid produced under conditions of an accumulation of this gas which brings about a progressive reduction in the amount given off. Although the results in such a case are quite comparable within themselves, it was felt that the repetition of the experiments, using a method by which the accumulation of carbon dioxid is prevented, was desirable. For these reasons further work on the rate of respiration of buds, flowers, young fruits, growing tips, and old shoots of two widely grown varieties, Early Black and Howes, has been performed. The work was done at the Massachusetts State Cranberry Substation at East Wareham, Massachusetts, during the summer of 1923.

## MATERIALS

The material used in this work consisted of buds, flowers, young and old shoots, and fruits of different ages, varying from very young to half grown, of two varieties of cranberries, Early Black and Howes. The material was collected and placed in the bottles for aspiration as promptly as possible. The collection of buds, flowers, and very young fruits proved to be very slow, so that 1½-2 hours usually elapsed before sufficient material could be obtained.

The Early Blacks were nearly through flowering when the Howes were in their prime. For this reason it was much more difficult to find buds of the Early Blacks. Only one lot of buds of this variety was collected. The buds used were fully grown and would normally have opened in 1-2

days. The young shoot material included the growing tip and 1-2 cm. of the current season's growth. Old shoots included 4-6 cm. of the leafy stems of the previous year's growth. Young fruits were usually taken 1-3 days after the petals had fallen and were generally 1-2 mm. in diameter and dark green in color. Fruits designated as "medium" were usually 3-4 mm. in diameter, but still showing a decided green color. The large fruits were 4-6 mm. in diameter, one third or one half grown, and pale green to whitish in color. It was not possible to select these sizes rigidly, but in picking the attempt was made to maintain these limits with some accuracy.

### METHOD

A titrimetric method was employed, using an apparatus similar in principle to that of Osterhout (10, p. 17). With the apparatus used it was possible to run determinations on four lots of material at one time. Uniform quantities by weight of material (usually  $\frac{3}{8}$  oz. = 10.6 g.) were collected and transferred to bottles of approximately 300-cc. capacity. The bottles were wrapped with heavy brown paper to exclude light and closed with 2-hole rubber stoppers. A vial of about 25-cc. capacity, also closed with a 2 hole rubber stopper, was connected by means of glass and rubber tubing with each bottle. A rubber bulb with a valve at each end was connected between the vial and the bottle containing the material, thus forming a closed system. A quantity of N/10 barium hydroxid, usually 5 or 10 cc., was placed in each vial. The tube leading from the bottle containing the plant material to the vial was drawn out to a taper and brought nearly to the bottom of the vial so as to be well under the hydroxid. By compressing and releasing the bulb the air was forced through the hydroxid. The aspiration period varied usually from thirty minutes to an hour, but was sometimes continued for one and one half to two hours. At the end of the period the amount of hydroxid consumed was determined by titration with N/20 hydrochloric acid. From this the amount of carbon produced, by volume, was calculated to standard conditions. The end point of the titration was determined to the nearest tenth of a cubic centimeter, which was considered sufficiently accurate in this work.

In titrating at the end of an aspiration period, the titration was made in the vial with whatever quantity of barium carbonate happened to be present. In order to determine whether or not the carbonate affected the titration, a number of tests were made. Equal quantities by weight of the same kind of material were run in pairs for periods of one half or one hour. At the end of the period an excess of 10% barium chlorid was added to one vial of the pair before titrating. The other vial of the pair was titrated without the addition of barium chlorid. In other tests made in the same way, the carbonate was allowed to settle, after which time the contents of each vial in the pair was divided into equal portions: one of clear solution and the other containing the carbonate. These portions

were then titrated separately. These tests were repeated two or three times. The titration values in all cases were the same. As the procedure of titrating directly in the vial containing barium carbonate without the addition of barium chlorid gave the same results as when chlorid was added and the clear supernatant solution pipetted off, the former procedure as the simpler was followed.

Temperature was not controlled. The variation within the duration of the experiment was usually not more than  $2^{\circ}$  C. The temperature on different days also showed but little variation, so that the results are quite comparable. In a few instances, deviations due to temperature were observed. In any case, lots of material run on the same day are strictly comparable as they were under the same conditions.

In comparing the respiratory activity of various parts of the plant and in presenting the results it has been found convenient to use the rate as the basis of comparison. The production of 6 cc. of carbon dioxid in one hour has been taken as the standard rate. The relative rate may be determined by reading the amount of carbon dioxid produced per hour, from which the time required to produce the standard amount, 6 cc., is calculated. The standard time divided by the value thus obtained gives the relative rate. Or, since the amount of carbon dioxid produced is essentially, if not actually, a linear function of the time, the relative rate may be obtained more directly by dividing the amount produced per hour by the standard amount per hour, *i.e.*, 6 cc. In the graphs the rate may be read as cubic centimeters per hour or as a percentage of the standard rate. The points on the graphs represent the average rate for the period during which the measurement was made and are located in the middle of the period.

## RESULTS

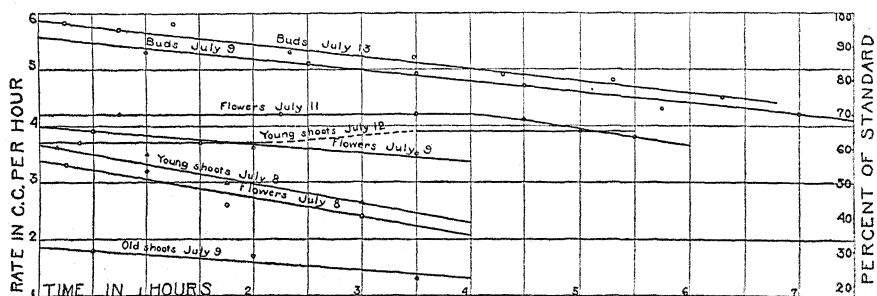
The results are shown graphically in text figures 1-7. In determining the rates, in as many cases as possible, two or more experiments using the same kind of material were run. In some experiments the comparison was made with different parts of the plant of one variety. In other experiments the relative respiratory rate was determined by a comparison of corresponding parts of the two varieties used.

In plotting the results it has been found necessary in several instances to place the graphs representing the activity of certain lots of material run on the same day in different figures to avoid confusion of lines, because of similarity in the respiratory activity of the parts used.

## DISCUSSION OF RESULTS

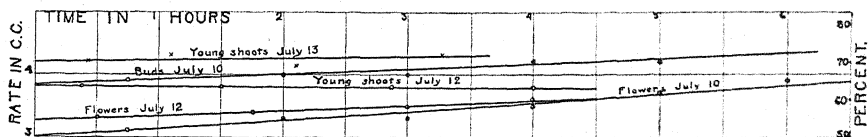
These experiments show that the parts of high respiratory activity are the buds, young shoots, and young fruits for a period of one to three days after the petals drop. The buds and young fruits were found to be more

active than young shoots in all but one instance. The exception was in the case of young shoots of Howes on July 9. In this experiment the respiratory rate of young shoots equaled that of buds of the same variety.



TEXT FIG. 1. Graphs showing the rate of respiration of buds, flowers, and young and old shoots of cranberries of the Howes variety on different dates. The temperature in all cases varied from 23° to 25° C. except for the two lots on July 8, which were at 19° C.

As only one experiment to determine the respiratory rate of buds of Early Blacks was performed, this experiment must serve as the basis of comparison of the relative activity of buds of Early Blacks and Howes. Making the comparison with the buds of Howes on July 13 (text fig. 1), it may be seen that at the beginning of the periods the rate of respiration of the buds of Howes was 1.5 cc. per hour, or 25% of the standard rate, higher than that of the buds of Early Blacks on July 10 (text fig. 2). The respiratory rate of buds of Howes on July 9 (text fig. 1) was less than that on July 13 by 0.2-0.3 cc. per hour.

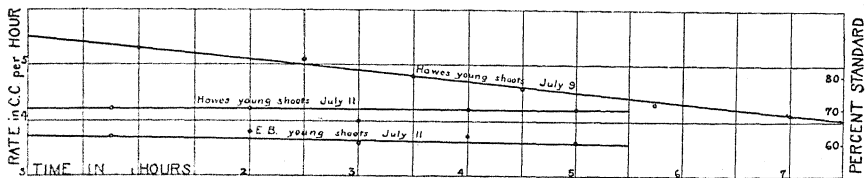


TEXT FIG. 2. Graphs showing the rate of respiration of buds, flowers, and young shoots of Early Black cranberries on different days. The buds and flowers on July 10 were run at a temperature of 21.5-23.5° C., the other lots at 23-25° C.

There was a difference of 2.5° C. in the temperatures at which the rates in the two varieties were determined, the Early Blacks having been at the lower temperature. Near the ends of the periods the respiratory rate of the buds of Early Blacks approaches that of the Howes. The temperature at this time was practically the same for both lots. If the experiments had been continued, the rates would probably have run closely parallel. On the assumption that the respiratory rate increases by 2-2½ times with a rise in temperature of 10° C., as has been shown by various investigators, the initial difference in the observed rate would be explained by the difference in temperature. It seems fair to assume, therefore, that the rate of respiration of buds of Howes and of Early Blacks is essentially the same.

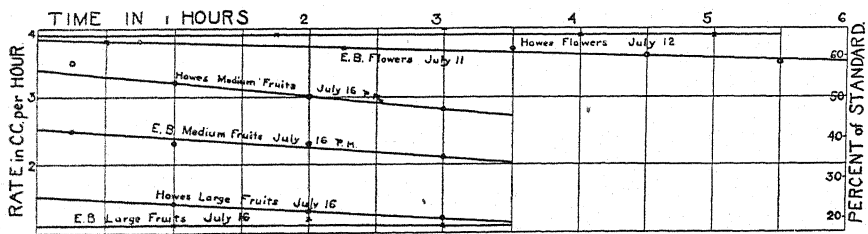


The rate of respiration of different lots of flowers of the same variety on different days shows considerable variation. This is probably due to



TEXT FIG. 3. Graphs showing the relative rate of respiration of young shoots of Howes and of Early Blacks on July 11, and of young shoots of Howes on July 9. Temperature July 9,  $24^{\circ}$ ; July 11,  $22.5-24^{\circ}$ .

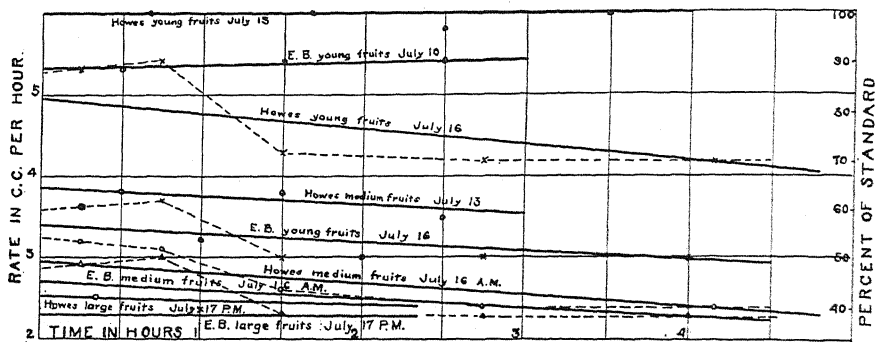
lack of uniformity of the material selected. The rate as measured represents the average activity of a large number of individuals comprising the lot. In selecting flowers or buds, individuals of the same approximate age and activity are not always secured. Hence the measured rates of two or more lots of material on different days show more or less variation. In spite of these variations, the experimental results throughout indicate a slightly higher rate for flowers of Howes than for those of Early Blacks. The differences are shown more clearly in direct comparisons, as on July 11 (text figs. 1, 4) and on July 12 (text figs. 2, 4). These differences are small but consistent, and are probably due to a difference in the relative maturity of the flowers in the two varieties. The average age of the flowers of the Early Blacks was probably somewhat greater than that of the Howes. The former variety had nearly reached the end of its flowering period while the Howes were at their best.



TEXT FIG. 4. Graphs showing the relative rate of respiration of flowers and of medium and large fruits of Howes and Early Blacks. The rate in the case of the medium and large fruits was determined at a temperature of  $20-23^{\circ}$  C., of the flowers on both dates at  $23-25^{\circ}$  C.

The effect of temperature on the rate of respiration is shown in the determination of the rate in flowers on July 8 (text fig. 1) and on July 10 (text fig. 2). The temperature during the period of respiration on July 8 was  $19^{\circ}$  C. On July 10, at the beginning of the period the temperature was  $21.5^{\circ}$  C. In the other experiments the temperature remained at  $23-25^{\circ}$  C. By making allowance for these differences in temperature, the

discrepancy in the rate of respiration as determined on different days is greatly reduced.

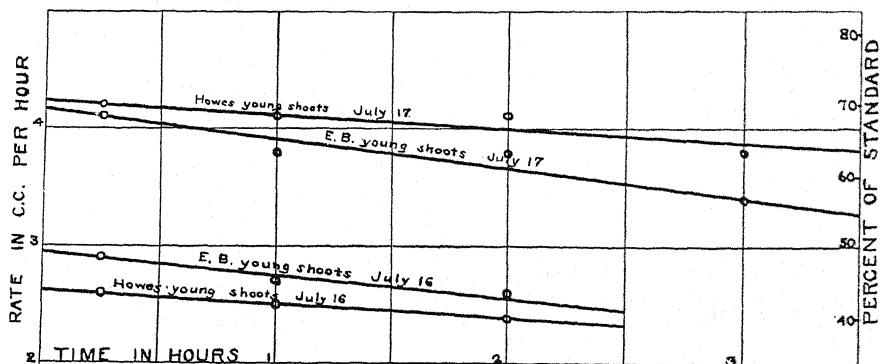


TEXT FIG. 5. Graphs showing the rate of respiration of young, medium, and large fruits of Early Blacks and Howes on different days. Temperature on July 10, 22.5–23.5° C.; on July 13, 23–25° C.; on July 16, 22.5–24.5° C.; on July 17, P.M., 22–23° C. An abrupt change in the rate of respiration of all lots run on July 16 occurred for which no explanation can be offered. No change in temperature was observed.

The respiratory activity of flowers is much less than that of buds. This is in agreement with the observations of Maige (7), who found that flowers just after opening respire less rapidly than the buds and that large buds are less active than small ones in the majority of the species studied.

The difference between the respiratory activities of buds and flowers is greater in Howes than in Early Blacks. In the former there is a difference of 1–1.5 cc. per hour in the rate of carbon-dioxid production, while in the latter variety it amounts to only 0.5–0.6 cc. per hour. This difference may be due to the fact that the buds and flowers of Early Blacks were the last of the season and therefore not thoroughly representative.

The rate of respiration of young shoots as determined experimentally shows considerable variation when considered with reference either to lots



TEXT FIG. 6. Graphs showing the relative rate of respiration of young shoots of Howes and Early Blacks on different days. The temperature during the experiment on July 16 was 19.5–21.5° C.; on July 17, 23–24° C.

of material of the same variety on different days or to the two varieties run at the same time. In two experiments, one on July 11 (text fig. 3) and the other on July 17 (text fig. 6), the relative respiratory rate of young shoots of Howes was greater than that of Early Blacks run at the same time. The temperature on these days was the same, and on any one day the lots compared were run at the same time. The difference in these instances must then be due to the material.

In one instance, on July 16 (text fig. 6), the rate of respiration of young shoots of Early Blacks was found to exceed that of Howes. On July 13 (text fig. 2) the rate was the same as that of Howes on July 11 (text fig. 3), both lots having been kept at the same temperature. The rate of respiration of young shoots of Early Blacks on July 12 (text fig. 2) was practically the same as that of Howes on the same date (text fig. 1).

The variation in the rate of respiration of different lots of shoots of the same variety on different days is due to the difficulty of selecting shoots of the same length. The measured activity of a lot of shoots depends upon the length of the shoots taken. The younger portions are the most active. If shoots uniformly 1 cm. in length from the growing tip were taken, the rate would be greater than if 2 or 3 cm. were used. In this work the tips were not selected to a uniform length, but varied usually between 1 and 2 cm. Hence lots of material picked at different times may be expected to show more or less variation aside from that due to differences in the temperatures at which the experiments were run. From the data at hand it must be concluded that there is no essential difference in respiratory rate between young shoots of the two varieties under similar conditions. The differences observed are to be attributed to lack of uniformity of the material selected.

Young shoots respire much more actively than old ones. On the basis of the rate determined for a single lot of old shoots of Howes it appears that young shoots respire two to three times as rapidly as old ones. This is in accordance with results obtained previously (1, p. 56) with Early Blacks. Garreau (6) has shown that for equal amounts of material, on the basis either of fresh or of dry weight under the same conditions of temperature in equal times, young shoots produce about twice as much carbon dioxid as those fully developed. Nicolas (9) found that in *Olea europea* L. the leaves of the current year produce a little more than twice as much carbon dioxid per hour per gram of material as the leaves of the year preceding. In *Quercus coccifera* L. young leaves of the current season were found to produce a little more than three times as much carbon dioxid per gram of material per hour as the leaves of the preceding year.

Very little difference was found in the relative activity of young shoots and of flowers either in Howes or in Early Blacks as determined in these experiments. On July 11 the rate of respiration was found to be the same in flowers and young shoots of Howes (text figs. 1, 3) and also in Early

Blacks (text figs. 3, 4). In Howes on July 12 the flowers showed a slightly higher rate during the first part of the aspiration period, but the rates became identical in the latter part of the period (text figs. 1, 4). Young shoots of Early Blacks on the same date were about 0.5 cc. per hour more active than the flowers. The latter showed a slight increase during the period of the experiment. At the end of the period the difference was only 0.2 cc. per hour (text fig. 2). The young shoots of Howes of July 8 were found to be slightly more active than the flowers run at the same time (text fig. 1). A somewhat greater difference in favor of the young shoots was found with Howes on July 9 (text figs. 1, 3). The higher respiratory rate of flowers and shoots on July 9 as compared with that of July 8 is due to difference in temperature. The material of July 8 was run at 19° C. On July 9 the temperature was 23–24° C.

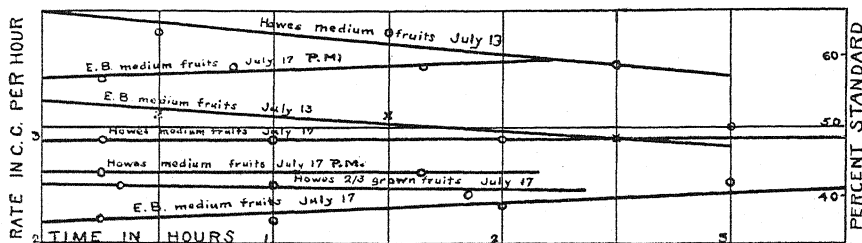
The relative rates of respiration of flowers and young shoots as determined by these experiments are quite consistent with the rates determined in former experiments (1, p. 56). In the former experiments the flowers were slightly more active than the shoots (both Early Black variety) except in one instance. In the present experiments the shoots are found to be more active, where any difference exists, both in Howes and in Early Blacks. The differences are small and may be explained readily by differences in the material selected.

Young fruits, for a period of 1–3 days after the petals have fallen, respire very rapidly, the rate equaling or exceeding that of buds. The highest rate observed was found in young fruits of Howes on July 13 (text fig. 5). Except in the very first part of the aspiration period, the young fruits on this date showed an activity of 0.7–1.0 cc. per hour greater than that of buds on the same day (text fig. 1).

The respiratory rate decreases rapidly with the development of the fruit. The decline in activity is not as evident when lots run on different days are compared as when the comparison is made among young, medium, and large fruits run on the same day. Thus, in the case of young and medium fruits of Howes on July 16 (text fig. 5), the rate of respiration of the former exceeds that of the latter by about 2 cc. per hour or 30% of the standard rate. Similarly, the respiratory rate of young fruits of Early Blacks is greater than that of the medium fruits on July 16 (text fig. 5) although the difference in this case is not as great, being only about 0.6 cc. per hour, or 10% of the standard rate, more than that of the medium fruits. The medium fruits of Early Blacks and Howes on July 16 (text fig. 4) and on July 17 P.M. (text figs. 6, 7) likewise showed a higher rate of respiration than the large fruits run at the same time.

In determining the respiratory rates of materials on July 16 (text fig. 5), a marked drop in the rate was observed from the second to the third period. The decline was proportional to the activity of the part used. The similarity in the change of rate in the four lots of material used makes it seem

improbable that a leakage or error in titration could have occurred. There was no change in temperature. No explanation can be offered for the sudden change in the rate. The graphs have been drawn to show the average rate during the entire period of aspiration. The points indicating the times at which measurements were made are connected by broken lines.



TEXT FIG. 7. Graphs showing the relative rate of respiration of young, medium, and large fruits of Howes and Early Blacks on various days. Temperature during determination of rate on July 13, 24–25° C.; on July 17, for Early Black and Howes medium fruits 23–24° C., for P.M. determinations and Howes 2/3 grown 22–23° C.

Some discrepancy occurs in the measured rates of respiration of materials of the same kind on different days, *e.g.*, of young and medium fruits of Howes on July 13 and 16 (text fig. 5); or of young fruits of Early Blacks on July 10 and 16 (text fig. 5). Such discrepancies are due to lack of uniformity in the development of the fruits selected. The fruit reaches practically its full size within 10 to 15 days after blossoming. It is difficult to pick fruits of corresponding stages of development, and any deviation in the selection of fruits as to size and age makes a very evident difference in the observed rate of respiration of the material used.

In comparing the rate of respiration of Early Black and Howes fruits at corresponding ages, the experimental evidence is somewhat contradictory. In general, the results indicate a rather higher rate for Howes than for Early Blacks at any age at which the rates were determined. On any given date, with materials run at the same time, the respiratory rate of Howes is, with one exception, clearly higher than that of the Early Blacks at a corresponding age (text figs. 5, 7). The exception is in the case of medium fruits of Howes and Early Blacks on July 17 P.M. (text fig. 7). In this instance the Early Blacks produced 0.8–1.0 cc. more carbon dioxide per hour than the Howes. Young fruits of Early Blacks on July 10 (text fig. 5) were found to respire at a rate only 0.6 cc. per hour less than that of Howes young fruits on July 13. The Early Blacks were at a temperature 2° C. lower than the Howes, which fact is sufficient to account for the difference in this case.

The difference in relative activity is greater in young and medium fruits of the two varieties than in the larger ones. This, with the variations found in the rate of respiration of the same kind of material as determined

on different days at corresponding temperatures, indicates that the higher rate found for fruits of Howes is due to the failure to select fruits of the same relative age in the two varieties. The fruits of Early Blacks, the earlier, faster-growing variety, tend to be somewhat older on the average, and hence show a lower rate of respiration than the fruits of Howes selected as of the corresponding age.

The results show two periods of maximum respiratory activity in the development of the flower, one in the bud stage and the other in the young fruit just after the petals have fallen. The difference in the respiratory activity of buds and flowers in the two varieties studied has been discussed (p. 646). The lower rate in the opened flower is due to the fact that the stamens more particularly and the corolla have reached their maximum growth while the pistil remains for a while unchanged. G. Maige (8) has shown that in each organ of the flower the respiratory intensity decreases with age, excepting in the pistil, which in some cases shows an increase.

In cranberries, soon after pollination the rate of respiration of the pistil increases to the second maximum. The carbon-dioxid production at this time is generally equal to that of buds just before opening. This period is of short duration; after it the rate of respiration declines rapidly with the development of the fruit. G. Maige states that in some cases the rate of respiration in the grown pistil is several times greater than at the earlier stages.

From the well known fact that the rate of respiration decreases with the reduction in the amount of carbohydrates in excised plant parts, it is to be expected that the lines representing the relative rates of the various materials used would show a downward trend during the course of the experiment. This is true in the greater number of instances, as may be seen by inspection of the graphs. In these cases the temperature has remained constant within one degree during the experiment. In several instances the rate of respiration was found to remain constant or to increase slightly during the experiment. This is the result of an increase in temperature of 2-3° C. In a few cases the experiments were too short to show any change in rate. The decrease in the rate of respiration per hour of duration of the experiment is very nearly the same in all kinds of material, whether run at the same time or on different days, provided that the temperature remained uniform.

#### CAUSE OF FLOODING INJURY

The injury to young shoots, buds, and blossoms of cranberries has been shown (1) to be correlated with oxygen deficiency. The exact nature of the injury resulting from such deficiency is not known, but the death of tissues is observed to occur when they are deprived of oxygen for some time. The oxygen necessary for the maintenance of life processes in the cranberry plant, under ordinary conditions, is obtained from the air. As long as

the plants are exposed to it, an unlimited quantity of oxygen is available at all times. When submerged, the supply is limited to the quantity in solution. This amount is very small in comparison with that in an equal volume of air. It is not the actual quantity of oxygen present, however, that determines whether or not injury will occur. This depends upon the ratio between the oxygen content of the water and the respiratory demands of the various parts of the plant. As long as the oxygen content of the water equals or exceeds the requirements of the plant, no harm will result.

#### FACTORS AFFECTING THE OXYGEN CONTENT OF WATER

The quantity and distribution of oxygen in the water is conditioned by a number of more or less closely interrelated factors. These may be classified in two groups: physical and biological. All factors are not necessarily in operation at the same time or always at the same rate. The oxygen content at any given time is the resultant of the number of factors acting and the rate of their operation.

##### Physical Factors

Physical factors determine the capacity of water to absorb or retain gases or affect the distribution of a gas. The factors concerned are: solubility, diffusion, currents, and wave action.

The quantity of any constituent of the air entering into solution in water depends upon the solubility of the gas and the partial pressure exerted by it. An increase of pressure causes more gas to go into solution. As ponds or reservoirs from which flooding water is drawn are usually shallow, increased pressure due to depth does not come into consideration. Moreover, the water is pumped or drawn off near the edge where the water as a rule is shallow, and in passing through the ditches on to the bog the water soon reaches a condition of equilibrium under atmospheric pressure, if not in that condition initially. The atmospheric pressure in a given locality undergoes only slight changes from day to day. For this reason the amount of gas entering into solution in water is essentially a function of temperature only. The solubility of gases in water varies inversely as the temperature, as shown by Pettersson and Sonden (11) and Roscoe and Lunt (12).

Carbon dioxid is much more soluble than oxygen, and the quantity in solution changes rapidly with a change in its partial pressure in the air. Under ordinary conditions this is essentially uniform, so that no variation in the amount in solution in water is induced on that account.

The distribution of gases in water is accomplished by the natural processes of mixing by wave action, convection currents, and diffusion. If the water is thoroughly mixed, an equilibrium between the gases in solution and those in the atmosphere is maintained. Wave action is the

most effective, as it stirs the water up, bringing it in contact with the air. The effectiveness of this method depends upon the velocity of the wind. In the absence of wind the cooling of the surface water establishes convection currents which to some extent equalize the distribution of the gases in solution.

Local conditions of supersaturation or deficiency of oxygen or carbon dioxid may occur when there is little or no wave action. A deficiency in the oxygen content of the water in reservoirs, ditches, and bogs has been repeatedly observed both in Massachusetts and in Wisconsin. It has also been found that, in all instances observed, the oxygen content of water in reservoirs decreases during the night. Both of these facts indicate that diffusion and convection currents as agencies in the distribution of gases are not adequate to maintain an equilibrium between the gases in solution and in the atmosphere.

### Biological Factors

Biological factors do not affect the capacity of the water to absorb gases or control their distribution. They operate through processes which produce or consume oxygen. These are photosynthesis, respiration, and the oxidation of organic matter. As the gases consumed or produced by these processes are obtained from or given off into the water, the presence of submerged organisms or of organic matter is an important consideration with reference to the gas content.

The replenishment of oxygen by photosynthetic activity can take place only when the light is of sufficient intensity. Other conditions being favorable, it goes on more rapidly with stronger illumination. Brown and Heise (3) have shown that the increase in rate is not directly proportional to the increase in light intensity. Although other processes which consume oxygen may be in operation at the same time, the production of oxygen by photosynthesis, with adequate illumination, exceeds its consumption. For this reason an increase in the oxygen content of the water of reservoirs and ponds usually occurs during the day. This is shown in text figure 8. It has been found that the increase in oxygen content is less on cloudy days than on clear.

Respiration, unlike photosynthesis, is not restricted to plants and does not depend upon light. Evidence of respiration is obscured during the day by the more active process of photosynthesis, but it is nevertheless in operation. Respiration in animals is the same as in plants in its effect on the gas content of water, so that the presence of large numbers of aquatic animal organisms augments the effect of the vegetation present.

Organic matter as it decays uses up oxygen. Direct oxidation may account for a small amount of this, but the greater part is exhausted through the action of bacteria which decompose the organic matter. In either case the oxygen consumed must be withdrawn from the water. For this



reason the presence of organic matter may cause a reduction in the oxygen content of the water below its normal capacity at a given temperature. As carbon dioxide is produced by the decay of organic matter, an increase of carbon dioxide is observed with the reduction of oxygen.

The results of a few analyses of gas collected from ditches and ponds in Massachusetts are given in table 1 as an indication of the effects of the decomposition of organic matter. A burette or other collecting vessel was arranged with an inverted glass funnel near the bottom of the pond or ditch so that bubbles of gas coming up from the organic matter were directed into the collecting vessel. When filled the apparatus was taken into the laboratory for analysis.

TABLE 1. *The Composition of Gas Mixtures Produced by the Decay of Organic Matter on the Bottoms of Ponds and Ditches in Massachusetts*

Source	Amount of Gas Taken in cc.	Carbon Dioxide		Oxygen	Residue	
		cc.	%	cc.	cc.	%
Ditch, Gleason's Bog.....	82.3	19.5	12.8	0.0	71.8	87.2
Ditch, Gleason's Bog.....	83.4	10.4	12.5	0.0	73.1	87.5
Ditch, State Bog.....	83.4	2.9	3.5	0.0	80.5	96.5
Ditch, State Bog.....	82.5	3.3	4.0	0.0	79.2	96.0
Cedar Pond (Nymphaea).....	85.1	4.6	5.4	0.0	80.5	94.6
Cedar Pond, Aug. 1, 1918.....	73.2	3.1	4.2	0.0	70.1	95.8
Cedar Pond, Aug. 2, 1918.....	90.2	4.15	4.6	0.0	86.0	95.8
Cedar Pond, Aug. 3, 1918.....	91.9	3.9	4.2	0.0	88.0	95.8

In this table corrections have been applied, so that the figures represent the volume of dry gas at 0° C. and 760-mm. pressure. No analyses for methane were made, but the residue burned freely when ignited, thus indicating a high percentage of this gas.

The rate at which any of these biological processes go on is influenced by temperature. It has been shown by many investigators that respiration goes on very slowly at 0-1° C. and that the rate increases twice or more with a rise in temperature from 1 to 10° C. or from 10 to 20° C. Ewart (4, p. 401) states that in warm-temperate, subtropical, and water plants photosynthesis ceases between 0° and 2° C., while in cool-temperate, arctic, and alpine plants it continues slowly until the plants are frozen. Blackman (2, p. 283) calls attention to the fact that photosynthesis conforms to van't Hoff's rule for the acceleration of the rate of reaction with rise of temperature up to 25-30° C. Above this temperature other limiting factors come into operation, as he shows (p. 283). The failure of van't Hoff's rule to apply above 25-30° C. has little bearing here, as such temperatures are very rarely reached in the case of submerged plants. In the oxidation of organic matter the same rule applies, since the consumption of oxygen and the liberation of carbon dioxide come about chiefly in consequence of the respiratory activity of the organisms involved in the decomposition.

For the reason that respiration and the oxidation of organic matter are opposite to photosynthesis in their effect on the oxygen and carbon-dioxid content of the water, the quantity of either gas present depends on the relative activity of these processes. The replenishment of oxygen by photosynthetic activity occurs only when the light is of sufficient intensity. On the other hand, the oxygen-consuming processes are continually in operation. This results in daily periodic variations of the oxygen content in bodies of water in which submerged vegetation and organic matter are present. These variations are greater on clear days than on cloudy ones.

#### CONDITIONS UNDER WHICH INJURY MAY OCCUR

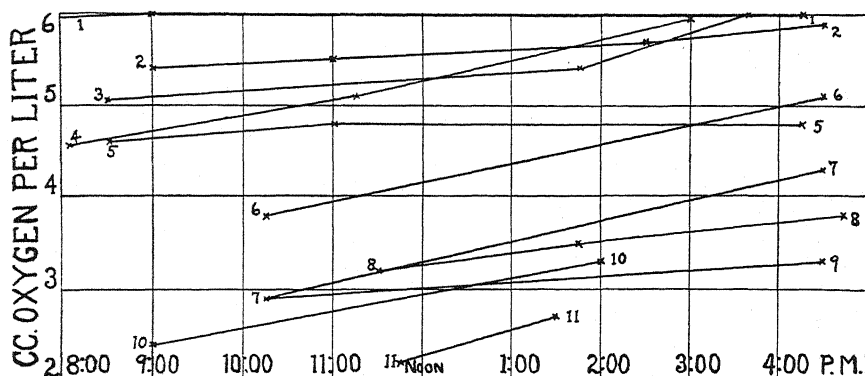
It has been shown that some factors operate to reduce oxygen-consumption, to increase its production, or to facilitate its replenishment. Other factors have an opposite effect. Injury occurs only when more oxygen is required than can be supplied. The degree of injury depends upon the extent to which the oxygen demand exceeds the supply and upon the duration of this condition. An analysis of conditions prevailing during a flooding period, with reference to the activity of the plants and other processes which consume oxygen or make its replenishment difficult, and to those which produce oxygen or facilitate its replenishment, is of value in determining whether or not injury is apt to occur and in ascertaining the factor or factors responsible for injury in case it occurs. All factors need not be adverse at the same time. On the other hand, a single adverse factor often has no visible effect, although a change in one factor may and often does involve another. For this reason the effect of a given factor can not always be stated with certainty. In flooding for protection against frost, for insect-control, for water-raking, or as a preventive against winter-killing, very diverse conditions exist.

Flooding for frost-control as ordinarily practised may be expected to cause little or no injury to buds, flowers, or young shoots in spring or to the fruits in fall, as in many cases it is necessary to hold the water only over night. If an abundant water supply is available, so that the bog may be flooded at night and the water drawn off the following morning, no serious injury is to be expected even during several days of cool weather with a daily recurrence of frosts.

When a lack of water makes it compulsory to hold a bog under flood for several days until the danger of frost is past, more or less injury may occur. Even in such a case the low temperature which prevails operates to reduce the amount of injury, as it retards the rate of respiration and hence lowers the oxygen requirement of the plants. At the same time the oxygen capacity of the water is increased and the consumption of oxygen by the oxidation of organic matter is greatly diminished.

In flooding for insect-control, low temperatures are much less apt to prevail. More prolonged flooding periods are necessary than in the case

of frost protection, and, as the plants are usually in an active condition when the flooding is done, injury occurs more frequently and is of a more serious nature. Very active parts such as young shoots, buds, flowers, and developing fruits are especially apt to be injured. The injury in this case is the result of a number of adverse factors working in combination. The higher temperature causes respiration and the oxidation of organic matter to go on at a more rapid rate. At the same time it decreases the capacity of the water for the absorption of oxygen.



TEXT FIG. 8. Graphs showing variations during the day in the oxygen content of water from various sources: 1, Spectacle Pond, East Wareham, Mass., June 29, 1919, clear, temperature 20-21° C.; 2, same, July 30, 1918, cloudy, temperature 23-26° C.; 3, reservoir, Beaver Brook, Wis., Sept. 8, 1919, cloudy, temperature 15° C.; 4, flooding section Beaver Brook Bog, Sept. 8, 1919, cloudy, temperature 15° C.; 5, Cedar Pond, East Wareham, Mass., Aug. 3, 1918, clear, temperature 24-26° C.; 6, Reservoir, Gebhardt's bog, Black River Falls, Wis., Sept. 11, 1919, clear, temperature 16-17° C.; 7, same, north end, Sept. 11, 1919; 8, same as 6, Sept. 10, 1919, cloudy, temperature 15° C.; 9, same as 7, Sept. 10, 1919; 10, Rogers' reservoir, East Wareham, Mass., Aug. 30, 1918, cloudy, temperature 19.5-25° C.; 11, reservoir, Walker, Wis., Sept. 20, 1919, partly cloudy, temperature 16-17° C.

As stated in other papers, light conditions just preceding and during the flooding period are important in determining the occurrence and degree of injury. With sufficient light, the production of oxygen by the photosynthetic activity of submerged plants compensates for the reduction in the capacity of the water to absorb oxygen which results from a rise in temperature. On a clear day, in water in which vegetation is present, the amount of oxygen produced by photosynthesis exceeds the amount consumed by respiration and by the oxidation of organic matter. For this reason an accumulation of oxygen occurs during the day even when organic matter is present (text fig. 8). This accumulation supplies the oxygen for respiration and for the oxidation of organic matter at a time when no oxygen is being produced and when, because of the slowness of diffusion, its replenishment from the air is not as rapid as its consumption. The

accumulation of oxygen during the day is often sufficient to satisfy all demands through a considerable part of the night. In this way injury from oxygen-deprivation is averted.

Although flooding injury is not apt to occur on clear days for the reasons indicated, it may come about on such days if there is no wind. The wind, even a gentle breeze, agitates the water and thus maintains a uniform distribution of oxygen. As the water reaches a depth of only a few inches over the bog, even a slight agitation is quite effective. On calm, clear days the only agencies that could operate to equalize the distribution of gases are convection currents and diffusion. As the water under these conditions is warmer at the surface, convection currents are not established. Diffusion is too slow to be effective. Hence, local deficiencies of oxygen arise in the vicinity of actively respiring parts and at the bottom where oxidizable organic matter is present. On the other hand, an accumulation of oxygen which may reach a condition of supersaturation arises in the vicinity of photosynthetically active parts or organisms. Buds and flowers consume oxygen very rapidly. This can not be replaced by diffusion from the water surface or from regions of high oxygen content as fast as it is consumed. Thus a local area of oxygen deficiency is created around the buds and flowers, causing injury to them. An instance of serious injury of this kind occurred on the State Bog at East Wareham, Massachusetts, in 1922.

Cloudiness sufficient to cause a retardation in the rate at which photosynthesis goes on reduces the amount of oxygen accumulating during the day. In times of dense cloudiness there may be no accumulation, or the oxygen content may decrease so that it becomes entirely depleted. This usually happens only when the cloudiness persists for two or more days, unless the bog has been flooded with water of low initial content. Under such conditions the injury from flooding is often serious.

The efficiency of flooding as a means of destroying insects depends upon holding the water for a sufficient length of time to drown them. The length of the period varies according to the nature and habits of the insect to be controlled. Little attention is paid to the temperature of the water, other weather conditions, particularly light, or to the character of the reservoir from which the water is drawn. As a result, injury is sometimes incurred when it might be avoided without interfering with an efficient control of the insects. Certain conditions lead to a low oxygen content of the water and thereby increase the probability of injury to plants. At the same time these conditions would be more effective in the drowning of insects, so that this could be accomplished in shorter time.

As an example of the effect of temperature on the length of time required to drown insect larvae, some experiments by Franklin (5, p. 230) may be cited. After describing the manner in which the experiment was performed, he states that:

In all these tests the sacks were of the same material, were tied up and submerged in the same way, to the same depth in the same place and for practically the same length of time. It will be seen that as the season advanced the submergence had much less effect on the worms. As the pond grew colder fast while these tests were in progress their results suggested that the temperature of the water largely determined its effect.

From other tests conducted later Franklin (5, p. 231) says:

The results of these experiments seem to prove that the effect of submergence of the worms in their cocoons depends largely, if not principally, upon the temperature of the water. . . .

The temperature effect observed by Franklin is due to the reduction in the oxygen requirement of the worms and to an increase in the oxygen content of the water as the temperature decreases. The respiration of insects is fundamentally the same as that of plants. The length of time required to drown insect larvae, therefore, is determined, not only by temperature which affects the rate of respiration and oxygen content of the water, but also by all other factors which influence oxygen content. Conditions which tend to reduce the oxygen content of the water and to increase the rate of respiration (oxygen requirement) of the larvae are most suitable for insect-control. At the same time, these conditions are most likely to be harmful to submerged plants. On this account it is necessary to regulate the length of the flooding period so that the greatest number of insects are killed with the least injury to the plants.

It has been observed in Massachusetts that injury from flooding occurs more often on bogs flooded with water from swamp reservoirs than on those for which the water is taken from ponds. In some instances in Massachusetts conditions are such that flooding over night causes injury. In nearly all cases the oxygen content of water in swamp reservoirs, both in Massachusetts and in Wisconsin, was below saturation at the temperature of the water at the time of sampling. The oxygen content of the water in swamp reservoirs varies greatly, depending on weather conditions, particularly as to cloudiness. During the day in clear weather the oxygen content may approach saturation. At night or on cloudy days the quantity of oxygen present becomes very small and may disappear entirely.

The most striking example observed in Massachusetts was that of a bog near Tremont. Duplicate samples of water from this reservoir taken on August 29, 1918, on the second day of a period of cloudy weather, showed an oxygen content of only 0.6 cc. per liter or 9.5% of the amount required for saturation at the temperature of the water (20° C.) at the time of sampling. A low oxygen content was observed in other swamp reservoirs also. One near Marion had, on a clear day, an oxygen content of 2.7 cc. per liter at 23.5° C. The oxygen content of the water in this reservoir on a cloudy day would probably be as low as that found in the reservoir at Tremont. The oxygen content of the water in Rogers' reservoir, near East Wareham, on August 30, 1918, is shown by graph no. 10, text figure 8.

Experimental evidence indicates that flooding injury is due to a deficiency of oxygen in the water. It is probable, therefore, that the injury to vines and blossoms on bogs flooded with water from swamp reservoirs is due to an oxygen deficiency caused by the presence of oxidizable organic matter in the reservoir. On this account the use of swamp reservoirs is objectionable. Although their use probably can not be avoided in most cases, the flooding period should be made as short as possible and special attention be given to weather conditions at the time of flooding.

### CONCLUSIONS

1. The rate of respiration of buds of Howes and of Early Blacks is essentially the same.

2. Variations in the rate of respiration of different lots of flowers of the same variety on different days are probably due to lack of uniformity as to age of the flowers selected.

3. The respiratory rate for the flowers of Howes is slightly higher than for those of Early Blacks. The average age of the latter was probably somewhat greater than that of the Howes.

4. The respiratory activity of flowers is much less than that of buds.

5. The difference between the rate of respiration of buds and that of flowers is greater in Howes than in Early Blacks. This difference may be due to the fact that the buds and flowers of Early Blacks were the last of the season and therefore not thoroughly representative.

6. Variations in the rate of respiration of different lots of shoots of the same variety on different days were observed. These were due to the difficulty in selecting shoots of the same length.

7. The data available indicate that there is no essential difference in the rate of respiration of young shoots of the two varieties under similar conditions.

8. Young shoots respire two to three times as rapidly as old ones.

9. Young shoots respire at about the same rate as the flowers in both the varieties studied.

10. Young fruits, for a period of one to three days after the petals have fallen, respire very rapidly, the respiratory rate equaling or exceeding that of buds.

11. The respiratory rate decreases rapidly with the development of the fruit.

12. The results indicate a slightly higher rate of respiration for Howes than for Early Blacks at any age at which the rates were determined. This is probably because the fruits of Early Blacks are somewhat older on the average than those of Howes selected as of the corresponding age.

13. There are two periods of maximum respiratory activity in the development of the flower: one in the bud stage, the other in the young fruit just after the petals have fallen.

14. Flooding injury occurs when more oxygen is required than can be supplied.

15. Low temperatures operate to reduce the amount of injury by decreasing the oxygen requirement of the plants and the consumption of oxygen in the oxidation of organic matter while at the same time increasing the capacity of the water to absorb oxygen.

16. Injury is less apt to occur on clear days because of the accumulation of oxygen from photosynthetic activity.

17. Flooding injury may occur on clear days in the absence of wind.

18. Conditions which reduce the oxygen content of the water and increase the oxygen requirement of the larvae are most suitable for insect-control. At the same time, these conditions are most likely to be harmful to submerged plants.

19. Flooding injury occurs more often on bogs flooded with water from swamp reservoirs than on those for which the water is taken from ponds.

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